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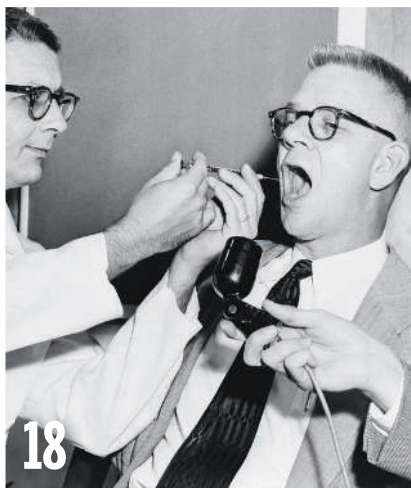
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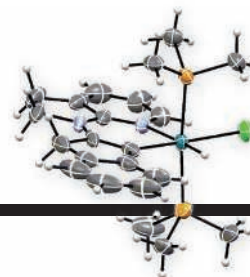
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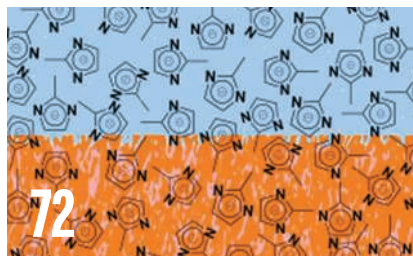
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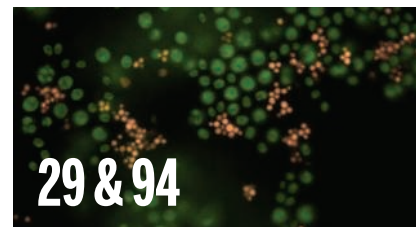
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ON THE COVER



Artist's view of a bilayer of graphene, in which two hexagonal carbon lattices are stacked on top of each other. The unusual band structure of bilayer graphene, coupled with external fields and interactions between electrons, leads to exotic quantum effects. See pages 31, 55, 58, and 61. *Image: C. Bickel/Science*

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Raising the bar

Numbers. Lots and lots of numbers. It is hard to find a paper published in *Science* or any other journal that is not full of numbers. Interpretation of those numbers provides the basis for the conclusions, as well as an assessment of the confidence in those conclusions. But unfortunately, there have been far too many cases where the quantitative analysis of those numbers has been flawed, causing doubt about the authors' interpretation and uncertainty about the result. Furthermore, it is not realistic to expect that a technical reviewer, chosen for her or his expertise in the topical subject matter or experimental protocol, will also be an expert in data analysis. For that reason, with much help from the American Statistical Association, *Science* has established, effective 1 July 2014, a Statistical Board of Reviewing Editors (SBoRE), consisting of experts in various aspects of statistics and data analysis, to provide better oversight of the interpretation of observational data.

For those familiar with the role of *Science's* Board of Reviewing Editors (BoRE), the function of the SBoRE will be slightly different. Members of the BoRE perform a rapid quality check of manuscripts and recommend which should receive in-depth review by technical specialists. Members of the SBoRE will receive manuscripts that have been identified by editors, BoRE members, or possibly reviewers as needing additional scrutiny of the data analysis or statistical treatment. The SBoRE member assesses what the issue is that requires screening and suggests experts from the statistics community to provide it.

So why is *Science* taking this additional step? Readers must have confidence in the conclusions published in our journal. We want to continue to take reasonable measures to verify the accuracy of those results. We believe that establishing the SBoRE will help avoid honest mistakes and raise the standards for data analysis, par-

ticularly when sophisticated approaches are needed. But even when taking added precautions, no review system is infallible, and no combination of reviewers can be expected to uncover all of the ways in which the interpretation of results may have gone wrong. In particular, it is difficult for reviewers to detect whether authors have approached the study with a lack of bias in their data collection and presentation.

I recall a study that I conducted years ago involving a global analysis of some oceanographic features that I was modeling to understand the rheology of oceanic plates on million-year time scales. I had only a handful

of data points—perhaps a dozen or so—and the fit to my model failed a significance test. Clearly, throwing out a few of the data points by declaring them “outliers” would have improved the fit dramatically, and in fact I even recall a reviewer of the paper commenting: “Can’t you make the data fit the model better?”

Really?

The editor published the paper despite the lousy fit of the model to the data. It was not too long before it was realized that those “outliers” were the key to a more complete understanding of the long-term rheological behavior of the oceanic plates. Although the model in the earlier paper needed an overhaul, the

fundamental observations, because they were presented without bias, inspired much further progress in the field.

In the years since, I have been amazed at how many scientists have never considered that their data might be presented with bias. There are fundamental truths that may be missed when bias is unintentionally overlooked, or worse yet, when data are “massaged.” Especially as we enter an era of “big data,” we should raise the bar ever higher in scrutinizing the analyses that take us from observations to understanding.

– Marcia McNutt



Marcia McNutt is Editor-in-Chief of *Science*.



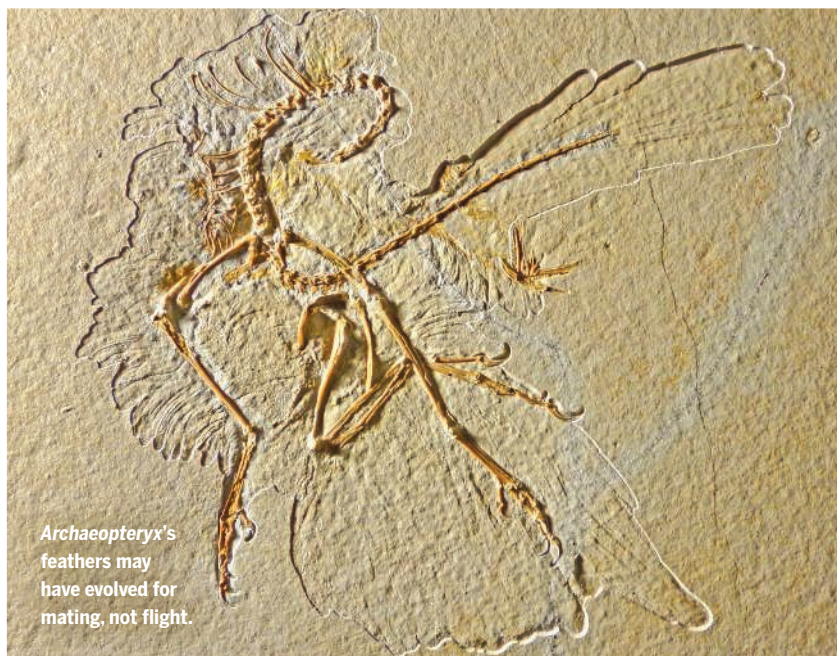
***“Readers must have confidence
in the conclusions published
in our journal.”***

“‘Genetically modified’ sounds Frankensteinish. ‘Drought resistant’ sounds really [like] something you want.”

Former Secretary of State Hillary Clinton, describing how genetically modified foods could have better spin, at the Biotechnology Industry Organization meeting in San Diego, California, on 25 June.

IN BRIEF

Feathers that didn't fly



To fly, modern birds now need a particular type of feather, with a thick stalk and a blade on either side. But researchers examining a new *Archaeopteryx* fossil think these “pennaceous,” or quill-like, feathers may have had other functions first, such as insulation or mating display. The fossil is an unusually complete specimen of the primitive bird, showing the quill-like feathers all over its body—not just on the wings and tail, but also on the body and legs. That body plumage would not have been aerodynamic, the team noted this week in *Nature*. Furthermore, fossils from other feathered dinosaurs and early birds show wildly varying distributions of pennaceous feathers—variations that make no sense if the feathers evolved to aid flight, the team concluded. “Aerial locomotion places fairly tight constraints on an animal; if there’s one fault in your design, you’re dead,” says paleontologist Oliver Rauhut of the Bavarian State Collection for Palaeontology and Geology in Munich, Germany. Instead, pennaceous feathers may have been later recruited for flying, once the creatures took to the skies. <http://scim.ag/archfeathers>

AROUND THE WORLD

Antibiotics to be focus of prize

LONDON | The public has spoken: The 2014 Longitude Prize, a new £10 million British award aimed at stimulating innovation, will go to whomever can “create a cost-effective, accurate, rapid, and easy-to-use test for bacterial infections that will allow health professionals worldwide to administer the right antibiotics at the right time,” the prize’s website states. The challenge was selected by the British public in a vote from six candidate themes (including dementia, paralysis, water, food, and flight) and was announced 25 June. The prize is a modern version of the £20,000 Longitude Prize, offered by the British government in 1714 for a method to determine a ship’s longitude at sea. Money for the 2014 version comes from the innovation charity Nesta and the government-funded Technology Strategy Board. Nesta and the Longitude Committee will now finalize the criteria for awarding the money; entries are welcome starting this fall. <http://scim.ag/Longitudeanti>

Europe to launch x-ray telescope

PARIS | The European Space Agency (ESA) announced 27 June that its next large space science mission will be the Advanced Telescope for High Energy Astrophysics (Athena), an instrument that will scan the universe for x-ray emissions from massive black holes and



other celestial objects. Athena is scheduled for launch in 2028 and would be the largest x-ray space telescope ever built. “Athena will revolutionize our view of black holes and cosmic structures filled with million degree gas. We really



MpalaLive! keeps an eye on hippos and other Kenyan wildlife.

Hippos: The Web's newest stars

Want to wake up to the sounds of the African savannah—the snorts of hippos, the squawks of vervet monkeys? Starting last week, the Mpala Research Centre and Wildlife Foundation in central Kenya began live-streaming the antics of African wildlife via a website (http://mpalalive.org/live_cam) that includes video feeds from a riverbank popular with hippos, as well as with elephants, zebra, and antelope. The view is of “our principle work site,” says Douglas McCauley, an ecologist from the University of California, Santa Barbara. “It’s spectacular to share our site with millions of people.” Inspired by a 3-year project to study hippos’ role in the ecosystem, the website is supported by \$500,000 from the Annenberg Foundation/Explore.org and includes an illustrated field guide to 88 local animals, interviews with researchers, and lesson plans for teachers. The researchers hope to harness citizen scientists to help record the hippos’ behavior.

BY THE NUMBERS

100
million

People that could be fed by growing crops on “land-grabbed” areas, regions in developing countries that have improved infrastructure due to foreign investment, according to a study in *Environmental Research Letters*.

4
million

Number of distinct viral proteins in nature, according to viral ecologist Matthew Sullivan, speaking at the American Society for Virology meeting in Fort Collins, Colorado.

19%

Projected decline in Antarctica’s population of emperor penguins by 2100, according to a *Nature Climate Change* study. The penguin colonies are threatened by shifting sea ice cover due to climate change.

need this to build a holistic picture of the observable Universe,” said Kirpal Nandra, director of the Max Planck Institute for Extraterrestrial Physics in Garching, Germany, and lead investigator of the Athena proposal, in a statement. The Athena team must now draw up a detailed design and budget before ESA gives the final go-ahead for construction, planned to begin in 2019.
<http://scim.ag/AthenaESA>

‘Secret Science’ bill advances

WASHINGTON, D.C. | Republicans say it’s just good government. Democrats say it would undermine good government.



EPA has taken heat over data used in its air pollution regulations.

Last week, the science committee for the U.S. House of Representatives voted along straight party lines to prevent the Environmental Protection Agency from taking any action unless its rule or policy is based on scientific data that are “publicly available” and capable of “substantial reproduction.” Dozens of scientific and public health organizations say the wording is ambiguous and a recipe for inaction. But its sponsor, Representative David Schweikert (R-AZ), says the Secret Science Reform Act (H.R. 4012) simply guarantees that “public data is used to make public policy.” It’s not clear if the full House will take up the bill, and there’s no counterpart in the Senate.



Australian scientists react to pending job cuts.

Scientists protest job cuts

Nearly 1000 scientists across Australia grabbed placards last week to protest pending job losses at the nation's leading research organization, the Commonwealth Scientific and Industrial Research Organisation (CSIRO). "Scientists are not known for rushing to the barricades," says Anthony Keenan of the CSIRO Staff Association, who adds that although staff members are concerned about job cuts at CSIRO, they are "dismayed" at the government's short-sighted approach to science. Job cuts at CSIRO are the direct result of the government's decision in May to slash AU\$115 million, or 16%, from the organization's budget over 4 years. The conservative government, elected last September, has also chosen not to appoint a science minister, the first time since the portfolio was created in 1931. After speaking at a protest in Canberra, former science minister and opposition Labor Senator Kim Carr blasted the government with this tweet: "No Science Minister, no policy, no idea." <http://scim.ag/CSIROprotest>

Sugar debate heats up

WASHINGTON, D.C. AND LONDON | Efforts to convince American and British consumers to eat healthier foods are, predictably, generating debate. In the United States, the Food and Drug Administration (FDA) last week held a public meeting to discuss changes proposed in February to its Nutrition Facts label, which summarizes the contents of food and beverages. Since

FDA last overhauled its food label in 1993, obesity rates, eating patterns, and understanding of various nutrients have changed substantially. FDA has so far received more than 4000 comments on its draft proposal. U.K. officials, meanwhile, want to recommend limits on how much added sugar consumers should ingest. The United Kingdom's Scientific Advisory Committee on Nutrition released a report last week suggesting that "free sugar"—which includes added sugars and some naturally present sugars—make up on average 5% of the country's energy intake. <http://scim.ag/dietsugar>

No quick fix for U.K. science ed

LONDON | There's a good reason that the Royal Society calls its new report on improving U.K. science and mathematics education a "vision" for 2030: There's no quick fix for a system that it says leaves most U.K. students without the necessary skills to be productive global citizens. Its wish list includes more math and science

for upper-level students, more teachers from the ranks of college graduates with science degrees, continued upgrading of the skills of current teachers, and a smaller role for high-stakes tests in assessing a student's progress. "Education is a political football," says co-author Julia Higgins, a professor emeritus of chemical engineering at Imperial College London. She hopes the report will convince policymakers "to relinquish some of their meddling powers ... [and] get the political parties to stop batting the thing back and forth." <http://scim.ag/UKstem>

NEWSMAKERS

Three Q's



Ten months after a chemical attack in Ghouta, Syria, the last 8% of Syria's known chemical arsenal left the country on 23 June. The shipment was a victory for the Organisation for the

Prohibition of Chemical Weapons (OPCW). But more needs to be done to make the world free of chemical weapons, says OPCW Director-General **Ahmet Üzümcü**. http://scim.ag/_Uzumcu

Q: How close are we to disarmament in Syria?

A: All declared chemical weapons are outside of the country. If there are still some other assets related to chemical weapons in Syria, we don't know. ... We are raising some questions about their declaration. [Then] I think we'll be able to say more confidently that the chemical weapons programs are totally eliminated.

Q: Only six countries have not joined the Chemical Weapons Convention. What happens when they do?

A: That will be an important achievement, but prevention of use of toxic substances will continue to be important, through the implementation of our convention but also through national measures.

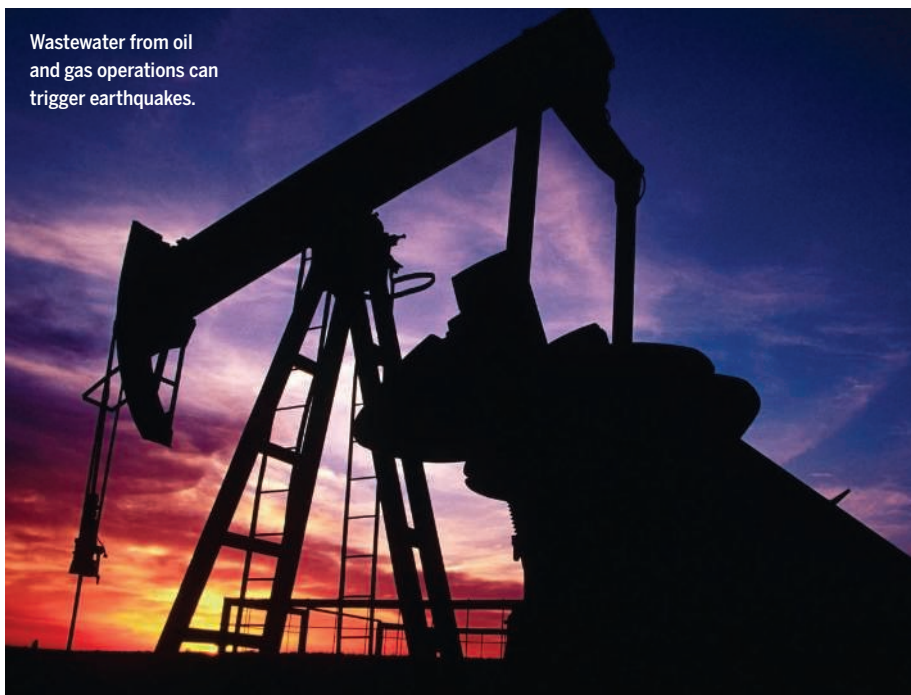
Q: How can scientists help?

A: Apart from our scientific advisory board ... we want to reach out ... to young generations, scientific communities, academia, to raise awareness. In many countries the penal code includes references to our convention and [misguided researchers] would have to be punished. Therefore, they have also to be aware of their obligations.



IN DEPTH

Wastewater from oil and gas operations can trigger earthquakes.



GEOSCIENCE

Injection wells blamed in Oklahoma earthquakes

Regulators in several states start to limit wastewater disposal to reduce risks of induced tremors

By Eric Hand

The town of Jones, Oklahoma, has one bar, three restaurants, and nine churches. Since 2008, it has also been home to a continuing swarm of 2547 small earthquakes—nearly one for each of the town's 2692 residents. Early on, when earthquakes were felt, people would call City Hall, says the mayor, Ray Poland. "They were scared and they wanted the mayor to do something. My smart-ass response was that I was going to put a stop to them."

Turning off Oklahoma's earthquakes is not so far-fetched an idea: Increasingly, scientists have linked them to nearby injection wells that are used to bury massive amounts of wastewater from oil and gas extraction operations. The pressure of that water in the pore space of rock can reduce the forces keeping a fault locked, and potentially trigger a rupture. The Jones swarm, however, has resisted such a con-

nection because there were no significant injection sites within 20 kilometers. But a study published online this week in *Science* (<http://scim.ag/Keranen>) shows how four of the state's most prolific wastewater wells could be responsible for creating a wave of pressure that crawled through the subsurface and triggered the earthquakes in the Jones swarm, some as far as 35 kilometers away. "The big distances here are the surprise," says William Ellsworth, a seismologist at the U.S. Geological Survey (USGS) in Menlo Park, California.

The study is yet another link connecting a surge of earthquakes across Ohio, Arkansas, and Texas to those states' hot pursuit of oil and gas. Among the lower 48 states, Oklahoma has become the unlikely earthquake king. Through 15 June, Oklahoma had seen 190 earthquakes magnitude 3 or greater in 2014, compared with 71 in California, the customary record-holder (see figure, right). "It's a concern," says Austin Holland, a seismologist at the Oklahoma

Geological Survey (OGS) in Norman.

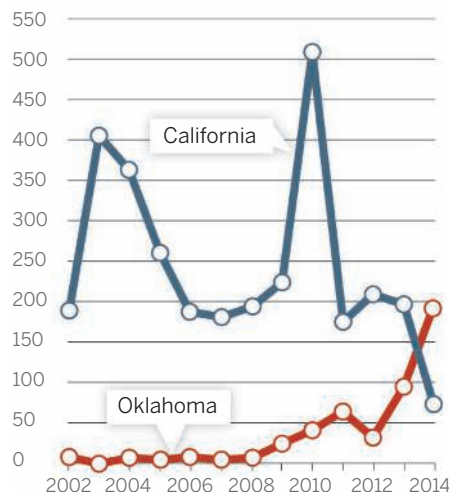
The problem, scientists say, is disposing of wastewater from water-intensive extraction operations. Hydraulic fracturing, or fracking, is partly to blame, but more concerning are disposal wells used in "dewatering" operations, in which oil and gas are separated out from huge amounts of water pumped up from a target rock formation.

The vast majority of Oklahoma's more than 9000 injection wells cause no trouble whatsoever. Not so with four high-volume disposal wells used in a dewatering operation near Oklahoma City, the study suggests. The wells pump more than 4 million barrels (477,000 cubic meters) of water into the ground every month. Katie Keranen, a geophysicist at Cornell University, and colleagues found that the four wells are capable of triggering the earthquakes. By combining precise maps of Jones swarm earthquakes with a hydrogeologic model, they showed that an expanding underground wave of pressure from the wells (named Chambers, Flower Power, Deep Throat, and Sweetheart) closely matched the places and times of the quakes in the swarm. The company that owns the wells, New Dominion, based in Tulsa, Oklahoma, declined to answer questions about the study and released a statement saying that it was based on "false assumptions."

Keranen is concerned that the four disposal wells lie close to the Nemaha fault, which runs through Oklahoma City and is large enough to host a devastating magnitude-7 earthquake. The main fault is unlikely to rupture because local stresses push

On shaky ground

Number of earthquakes, magnitude 3 or greater



Source: USGS

its two sides together, Keranen says, but an unmapped offshoot might be more susceptible to rising water pressures. In the long term, a magnitude-6 earthquake near Oklahoma City is a plausible hazard, she says.

Regulators are starting to get involved. On 20 June, Oklahoma's governor approved a new rule that, beginning later this year, would require oil and gas operators to report injection volumes and pressures daily rather than monthly. In September 2013, the Oklahoma Corporation Commission (OCC) exercised its regulatory powers for the first time in an induced seismicity case: It ordered a well operator in Love County to dial back disposal volumes after the well was connected to a magnitude-3.4 quake. Regulators in other states, such as Ohio and Arkansas, have taken similar actions. Just last week in Colorado, regulators ordered a 20-day halt to operations at an injection well in Weld County after it was linked to a magnitude-3.4 earthquake. OCC says that it is evaluating the new study in *Science* and is in discussions with New Dominion about the four high-volume wells.

Several unresolved questions loom large for researchers. For starters, they have a hard time identifying a safe rate of wastewater disposal because so much depends on the local geology. "We don't have a magic number at this point. Some of this is trial and error and experiment," says OGS's Holland.

Nor does anyone know whether the extraordinary bursts of small earthquakes could signal bigger ones to come. As a rule of thumb, seismologists estimate that for every 1000 magnitude-4 earthquakes, there will be 100 magnitude-5 quakes, 10 magnitude-6 quakes, and so on. So far, the largest injection-linked earthquake to occur has been the 2011 magnitude 5.7 in Prague, Oklahoma, which knocked off spires at a university 25 kilometers away. Are worse ones looming? "That's the key question that no one has an answer to at this point," USGS's Ellsworth says.

Researchers hope that better earthquake monitoring—and better injection well reporting—will help them come up with answers. Holland says that by the end of the year, the state plans to boost its number of permanent seismic stations from 17 to 25—still far behind California's 2530 stations.

Meanwhile, in Jones, earthquake talk has become a staple at Shuff's Main Street Grill. Joe Dooling, who curates the town's historical museum and whose home's ceiling was cracked during one of the early earthquakes, jokingly says the mayor had better be working on his campaign promise. "Ray has been duly warned that if he doesn't stop the earthquakes, we're not going to elect him next time." ■



A 2013 Supreme Court decision that barred human gene patents is scrambling patenting policies.

INTELLECTUAL PROPERTY

Biotech feels a chill from changing U.S. patent rules

Supreme Court decisions hobble efforts to protect inventions involving natural products

By Kelly Servick, in San Diego, California

A year after the U.S. Supreme Court issued a landmark ruling that human genes cannot be patented, the biotech industry is struggling to adapt to a landscape in which inventions derived from nature are increasingly hard to patent. It is also pushing back against follow-on policies proposed by the U.S. Patent and Trademark Office (USPTO) to guide examiners deciding whether an invention is too close to a natural product to deserve patent protection. Those policies reach far beyond what the high court intended, biotech representatives say.

"Everything we took for granted a few years ago is now changing, and it's generating a bit of a scramble," says patent attorney Damian Kotsis of Harness Dickey in Troy, Michigan, one of more than 15,000 people who gathered here last week for the Biotechnology Industry Organization's (BIO's) International Convention.

At the meeting, attorneys and executives fretted over the fate of patent applications for inventions involving naturally occurring products—including chemical compounds, antibodies, seeds, and vaccines—and traded stories of recent, unexpected rejections by USPTO. Industry leaders warned that the uncertainty could chill efforts to commercialize scientific discoveries made at universities and compa-

nies. Some plan to appeal the rejections in federal court.

USPTO officials, meanwhile, implored attendees to send them suggestions on how to clarify and improve its new policies on patenting natural products, and even announced that they were extending the deadline for public comment by a month. "Each and every one of you in this room has a moral duty ... to provide written comments to the PTO," patent lawyer and former USPTO Deputy Director Teresa Stanek Rea told one audience.

At the heart of the shake-up are two Supreme Court decisions: the ruling last year in *Association for Molecular Pathology v. Myriad Genetics Inc.* that human genes cannot be patented because they occur naturally (*Science*, 21 June 2013, p. 1387); and the 2012 *Mayo v. Prometheus* decision, which invalidated a patent on a method of measuring blood metabolites to determine drug doses because it relied on a "law of nature" (*Science*, 12 July 2013, p. 137).

Myriad and *Mayo* are already having a noticeable impact on patent decisions, according to a study released here. It examined about 1000 patent applications that included claims linked to natural products or laws of nature that USPTO reviewed between April 2011 and March 2014. Overall, examiners rejected about 40%; *Myriad* was the basis for rejecting about 23% of the applications, and *Mayo* about 35%, with some

overlap, the authors concluded. That rejection rate would have been in the single digits just 5 years ago, asserted Hans Sauer, BIO's intellectual property counsel, at a press conference. (There are no historical numbers for comparison.) The study was conducted by the news service Bloomberg BNA and the law firm Robins, Kaplan, Miller & Ciseri in Minneapolis, Minnesota.

The numbers suggest USPTO is extending the decisions far beyond diagnostics and DNA, attorneys say. Harness Dickey's Kotsis, for example, says a client recently tried to patent a plant extract with therapeutic properties; it was different from anything in nature, Kotsis argued, because the inventor had altered the relative concentrations of key compounds to enhance its effect. Nope, decided USPTO, too close to nature.

In March, USPTO released draft guidance designed to help its examiners decide such questions, setting out 12 factors for them to weigh. For example, if an examiner deems a product "markedly different in structure" from anything in nature, that counts in its favor. But if it has a "high level of generality," it gets dinged.

The draft has drawn extensive criticism. "I don't think I've ever seen anything as complicated as this," says Kevin Bastian, a patent attorney at Kilpatrick Townsend & Stockton in San Francisco, California. "I just can't believe that this will be the standard."

USPTO officials appear eager to fine-tune the draft guidance, but patent experts fear the Supreme Court decisions have made it hard to draw clear lines. "The *Myriad* decision is hopelessly contradictory and completely incoherent," says Dan Burk, a law professor at the University of California, Irvine. "We know you can't patent genetic sequences," he adds, but "we don't really know why."

For now, Kostis says, applicants will have to get creative to reduce the chance of rejection. Rather than claim protection for a plant extract itself, for instance, an inventor could instead patent the steps for using it to treat patients. Other biotech attorneys may try to narrow their patent claims. But there's a downside to that strategy, they note: Narrower patents can be harder to protect from infringement, making them less attractive to investors. Others plan to wait out the storm, predicting USPTO will ultimately rethink its guidance and ease the way for new patents.

USPTO has extended the deadline for public comment to 31 July, with no schedule for issuing final language. Regardless of the outcome, however, Stanek Rea warned a crowd of riled-up attorneys that, in the world of biopatents, "the easy days are gone." ■

RUSSIA

Plan to grade institutes rattles Russian academy

Researchers fear reforms mark path to institute closures

By Vladimir Pokrovsky

To researchers at the beleaguered Russian Academy of Sciences (RAS), the omens are dark. Last month, the government body that took over management of RAS property and finances in January published a road map for reform. Among the measures it calls for is a formal assessment of the research effectiveness of RAS institutes. Scientists have concluded that the only reason to grade institutes is to decide which ones to close down.

"Such suspicions have existed from the very beginning of this period of RAS reform," says physicist Andrey Tsaturyan of Moscow State University's Institute of Mechanics and co-chair of the Council of the Researchers Society, an organization of RAS scientists. The timing of the road map is especially alarming, he adds, given that a yearlong moratorium on any changes to the staff and property of RAS, set by President Vladimir Putin (*Science*, 6 December 2013, p. 1157), will soon come to an end. Meanwhile, a draft of a law setting an age cap for institute directors threatens to leave many centers leaderless and vulnerable.

Few dispute that RAS, which runs the country's major research institutes and flourished during the Soviet era, needs reform, but many academy scientists are wary of the government's approach. Under the road map, which appeared last month on the website of the Federal Agency for Scientific Organizations (FASO), the next 6 months will be spent devising criteria for the assessment, which will begin on 1 January 2015. Other measures will follow, including developing competitive funding schemes, upgrading equipment, raising publication activity, boosting researchers' qualifications, and transferring staff employment terms to a contract system.

The road map also proposes raising RAS researchers' salaries to twice the average in

the region in which they work. Researchers, who are often poorly paid, welcome the increase but wonder where the money will come from. The one cost-saving measure in the road map is a reduction in the proportion of technical and support staff from 50% to 41%. That worries biologist Viktor Krivokhatskiy of the RAS Zoological Institute in St. Petersburg, who says that "cuts in technical personnel would worsen the already miserable state of equipment and collections in Russia."

Researchers are also skeptical about FASO's intention to develop new criteria for assessing institutes. "There is no point in inventing new criteria of effectiveness and denying the only effective ones that are used in the world, relating to publications and research results," says Vasily Afonyushkin, a biologist at the Novosibirsk RAS Experimental Veterinary Institute.

Although the FASO road map doesn't mention closing down institutes, some are concerned that the same result will be achieved through stealth. In early June, the Russian Cabinet sent a draft law to the Duma setting an upper age limit of 65 years for institute directors and their deputies. According to RAS trade unions, half of RAS's 800 institute directors would have to stand down.

Such a measure will "behead the majority of such institutions and probably ruin

them," says academician Michael Ugrumov of the RAS Institute of Normal Physiology in Moscow. Because of the severe brain drain that afflicted Russia during the economic turmoil of the 1990s, there is a shortage of senior scientific managers with the skills to take over so many directorships, he contends. "I'm not saying the laboratory heads scenario is inevitable, but I see certain signals and believe that we have to prevent such a disaster." ■

Vladimir Pokrovsky is a writer in Moscow.

New problem for older directors

65

Proposed age cap for directors of RAS's institutes

50

Percentage of directors older than that age cap, according to RAS trade unions



INTERVIEW

A challenge to pseudoscience

Egyptian expat questions claims that devices noninvasively detect viruses in blood and treat infected people

By John Bohannon

Earlier this year, the Engineering Authority of Egypt's military announced a hand-held instrument that could detect a variety of viral infections without even touching a person, and another device that clears a patient's blood of viruses. Treatment of Egyptian patients with both devices was due to begin 30 June but 2 days before, the militia announced plans for "more testing" on the latter device. According to government officials, the devices will not only wipe out AIDS and hepatitis at home—Egypt has the highest prevalence of hepatitis C in the world—but will also make a fortune as foreign patients flock to the country. Whereas the Western scientific community has ridiculed the devices as pseudoscience, Egyptian academics have been largely silent. The country's military regime has been handing down harsh criminal punishments to its critics, including journalists. But one expat Egyptian scientist, Islam Hussein, has created videos explaining the devices' scientific problems, one of which has garnered more than 100,000 views on YouTube—a large number considering they are 80-minute Power-Point presentations in Arabic. Like most of Egypt's top scientific talent, Hussein, 36, left his country. After a virology Ph.D. at the University of Cambridge in the

United Kingdom, he settled at the Massachusetts Institute of Technology in Cambridge in the United States, where he researches avian influenza. This interview has been edited for clarity and brevity.

Q: What do you know about these devices?

A: The first is called C-FAST. [The Egyptian Armed Forces] claim that the antenna of this device can detect an infected patient from a distance of up to 500 meters. The device doesn't even need a battery; it is powered by the body's static electrical energy. The antenna supposedly detects the electromagnetic waves emitted by the vibrations of the hepatitis C viral genome in a sequence-specific manner. C-FAST is one of a big series of devices that detects several viral infections: HIV, hepatitis C, influenza, MERS coronavirus, and the malaria virus. Yes, malaria [caused by a protist] is now promoted to the rank of a virus.

The second is called the Complete Cure Device [CCD], which looks very much like a dialysis machine. It draws blood from the patient using a pump. The infected blood

passes through an expensive spiral tube—it is made of a very specialized material that its maker claims took 7 years to develop. The tube emits a mysterious radiation—it is a military secret—that kills the virus and then the blood is returned to the body.



Q: What makes them implausible?

A: For C-FAST, the research team has not presented any scientific evidence that electromagnetic waves emitted by viral nucleic acids are even detectable, let alone diagnostic. A discovery of this caliber deserves a *Science* or *Nature* paper. They claim that the electromagnetic signal of every virus is like a fingerprint that can be programmed onto a small chip inside the C-FAST device. By replacing a hepatitis C chip with one for influenza, a device becomes capable of detecting the flu-specific electromagnetic signal and so on.

As for CCD, we don't yet have a single scientific publication describing how this device is safe and effective. We have heard three conflicting mechanisms of action. Exposing a patient's blood to radiation will not rid him or her of hepatitis C replicating in the liver or HIV integrated into the genomes of infected CD4+ cells. They claim to have done experiments with chimps; they even claim that thousands of people were treated during a clinical trial. Again, where is the data?

Q: Are Egypt's scientists speaking out?

A: Essam Heggy, a research scientist at NASA's Jet Propulsion Laboratory and the former adviser of the ex-president of Egypt, issued a statement that this whole thing is a big scandal. He has been attacked by Egyptian media day and night. Very few Egyptian doctors have spoken out.

Q: What motivated you to make those videos?

A: I couldn't stand by watching this happen in my home country and keep quiet about it. This "cure" will affect millions of Egyptians, from the side effects of an unregulated, potentially toxic therapeutic device to false hopes that lead infected people to not take the necessary precautions.

Q: Are you worried about getting noticed by the government?

A: No, I am not worried. These videos were all about science and science only. There is no reason for the current regime or anybody at the Egyptian Armed Forces to get upset with what I have said.

Q: What impact do you hope to have?

A: Many people have made fun of the devices. However, nobody has taken the initiative to take them seriously and explain to the public why these claims are baseless. I was also hoping that my voice will reach the people behind these inventions and persuade them to change their course of action. ■

Faulty drug trials tarnish Japan's clinical research

Spate of scandals from postmarketing drug studies may compel reforms of clinical research practices

By Dennis Normile, in Tokyo

In a wearily familiar ritual, four University of Tokyo officials bowed deeply before assembled journalists on 24 June, apologizing for the school's role in a troubled clinical research project. Four days earlier, Takeda Pharmaceutical officials apologized for making false claims about a Kyoto University drug study. Last summer, Kyoto Prefectural University of Medicine and Tokyo's Jikei University School of Medicine officials were bowing, admitting that clinical studies conducted with Novartis Pharma, the Japanese subsidiary of the Swiss giant, had proven bogus. These and other revelations have shaken faith in Japan's clinical research efforts and shone a spotlight on the cozy ties between pharmaceutical companies and university researchers.

An overhaul of the nation's clinical research regulations and practices is overdue, says Iwao Kuwajima, chair of the Tokyo-based nonprofit Japanese Organization of Clinical Research Evaluation and Review. The ministry of health recently formed a clinical research advisory panel to produce recommendations to do just that. Reforms will be particularly important for realizing governmental hopes of making advanced medical technology an economic driver. Prime Minister Shinzo Abe's administration is laying plans for an agency akin to the U.S. National Institutes of Health (NIH) that will coordinate public and private efforts to turn basic findings into clinical applications (*Science*, 6 September 2013, p. 1053). Acceptance of any resulting therapies will hinge on having trustworthy scientific results, and the recent scandals "have seriously affected the credibility of clinical research efforts" in Japan, Kuwajima warns.

The apologies highlight problems in clinical studies of drugs already on the market. Manufacturers fund such studies hoping to show that their drugs cause fewer side effects or are more effective than competing products. In Japan, these studies are more loosely regulated than trials to demonstrate safety and efficacy.

The most egregious example of what has gone wrong is Diovan, a hypertension drug

that Novartis Pharma started selling in Japan in 2000. Beginning in 2002, Novartis sponsored clinical studies at five medical schools that all concluded that Diovan reduced the incidence of stroke, angina, and other complications better than competing medications. Novartis trumpeted these results—published in top journals—in its advertising. Sales hit \$1 billion in 2012.

But last year, investigations by two of the universities and the ministry of health found that the results had been rigged, allegedly by a Novartis employee who assisted

The committee found four other studies at the university in which Novartis employees were inappropriately involved. All have been halted.

Takeda's apology covered a postmarketing study of its hypertension drug Bopress, conducted by Kyoto University researchers. A third-party investigation commissioned by Takeda reported on 20 June that company promotional materials implied that Bopress had fewer side effects than a competing drug, although the results cited actually found "no statistical difference between the medicines."

"Pharmaceutical companies and researchers are too close," Kuwajima says.

There are other problems. A separate University of Tokyo report, also released on 24 June, on the Japanese Alzheimer's Disease Neuroimaging Initiative (J-ADNI) found no evidence of data manipulation, as had been alleged by a leading Japanese newspaper (*Science*, 17 January, p. 234). But the report called project management "inexperienced" and criticized its handling of data.

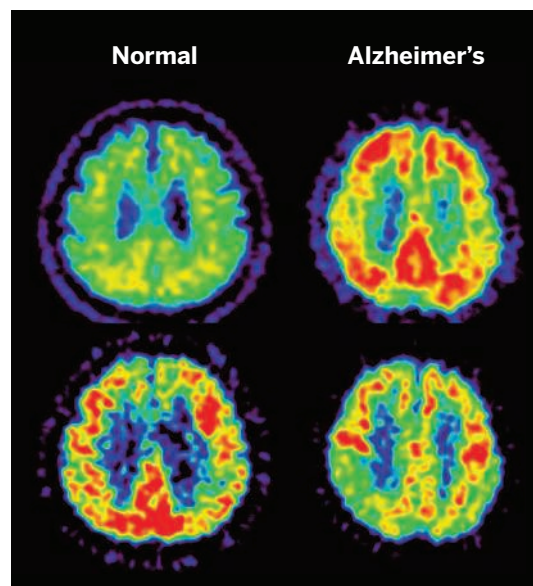
The \$31 million effort involving 38 institutions is "unprecedented" in scale and complexity for Japan, says Takeshi Iwatsubo, a University of Tokyo neuropathologist who heads J-ADNI. He acknowledges the project had trouble finding experienced data managers and biostatisticians, leading to a rocky start.

The manpower problems extend to regulatory agencies. "There are few people here capable of monitoring clinical research," says Kuwajima, who hopes that the NIH-like agency, coming next April, will fund training of clinical research managers as well as support research.

Tetsuya Tanimoto, a physician at the University of Tokyo who studies regulatory issues, says that while industry support for university research is welcome, "more trans-

parency and regulation are needed" to avoid conflicts of interest. He notes that in 2012, according to the Japan Pharmaceutical Manufacturers Association, member firms gave university scientists \$4.8 billion, an amount nearly double the budget of the education ministry's grants for individual researchers.

After bowing in apology last week, University of Tokyo Executive Vice President Yoichiro Matsumoto ruefully admitted that the problems show "a lack of awareness of research ethics." All of these shortcomings and more are on the agenda of the new health ministry advisory committee, which intends to issue a report this fall. ■



The J-ADNI project, criticized for poor data handling, uses PET scans to compare normal (left) and Alzheimer's diseased brains.

research teams with data analysis (*Science*, 19 July 2013, p. 223). The employee, who resigned from Novartis, was arrested last month and charged with violating Japan's Pharmaceutical Affairs Law, which bars hyping the benefits of medications. Prosecutors are investigating whether the company engaged in criminal conduct.

A University of Tokyo committee checking a postmarketing study of Novartis's leukemia drug, Tasigna, reported on 24 June that company employees had access to some of the findings as well as associated patient information, in violation of both the study protocol and of privacy laws.



Stephen Ross holding a chalice with a psilocybin capsule.

High hopes

Psychedelic drugs fell from grace in the 1960s. Now, scientists are rediscovering them as potential treatments for a range of illnesses

By Kai Kupferschmidt

In the back corner of a small locked room at the New York University College of Dentistry in New York City sits a 400-kilogram safe. Inside is a small plastic bottle holding a gram of a white powder with a bluish tinge. Every day, two people come into the room, open the safe, take out the bottle, and weigh it to make sure its contents are still there.

The powder is psilocybin, the active compound in hallucinogenic mushrooms. It's a schedule I substance, a category reserved for the most dangerous drugs. But Stephen Ross, the psychiatrist who had the safe installed, has been giving psilocybin to cancer patients since 2009. He believes it can reduce the anxiety and anguish of people facing death. His study could help pave the way for the first-ever phase III trial of psilocybin. If that succeeds, within a few years doctors might prescribe the drug for anxiety in cancer patients.

Treating people with psychedelic drugs may seem outlandish, but scientists have tried it before, in the 1950s and '60s. But then, the drugs' reputation took a sudden nosedive and the research "got stuck in a deep freeze for decades," says Roland Griffiths, a psychiatrist leading a psilocybin study at Johns Hopkins University in Baltimore, Maryland.

Now, more and more researchers are turning back to psychedelics, thanks to new funding and a fresh appreciation for their unmatched ability to alter the way the brain processes signals. "From a scientific standpoint, these are fantastically interesting compounds, completely unique in terms of their pharmacology and alteration in mood," Griffiths says. Psilocybin is used in studies to treat depression and obsessive-compulsive disorder and to help people quit their nicotine, alcohol, or cocaine addiction. Researchers are also testing LSD to treat anxiety or alleviate cluster headaches; ecstasy to treat post-traumatic stress disorder; and ibogaine—a substance from African plants—to treat opium withdrawal.

But this remains a contentious field, and as researchers rediscover these strange substances, they face difficult questions: Can they be safely given to patients with mental illness? Is the trip an essential part of the therapy or a side effect best avoided?

Should researchers try psychedelics themselves? And what can be learned from the drugs' controversial history?

THE PSYCHEDELIC ERA BEGAN with a bold self-experiment. On a Monday afternoon in April 1943, Albert Hofmann, a chemist at the pharmaceutical company Sandoz in Basel, Switzerland, took 250 micrograms of LSD. Hofmann had derived the compound from ergot, a fungus that grows on wheat, 5 years earlier in search of something to stimulate blood circulation and breathing, but it had shown few effects on rodents. Now, on a hunch, he decided to try it himself.

As Hofmann biked home, the world began to appear distorted and time seemed to slow. At home, the furniture turned into threatening forms, his neighbor appeared to him

PODCAST

To hear a podcast with author Kai Kupferschmidt, see http://scim.ag/pod_6192.

ducing extreme experiences that might lead to permanent changes in behavior. Others gave psychotherapy patients repeated low doses as a way of "loosening their minds." Researchers reported positive results in depression, anxiety, obsessive-compulsive behavior, and other disorders.

Most of the experiments were not up to modern standards, says neuroscientist Franz Vollenweider of the University of Zurich in Switzerland. Success was measured in vague terms, control groups were often lacking, or participants and experimenters weren't blinded. Still, the studies were very suggestive, and some have stood the test of time, says Robin Carhart-Harris, who studies psy-

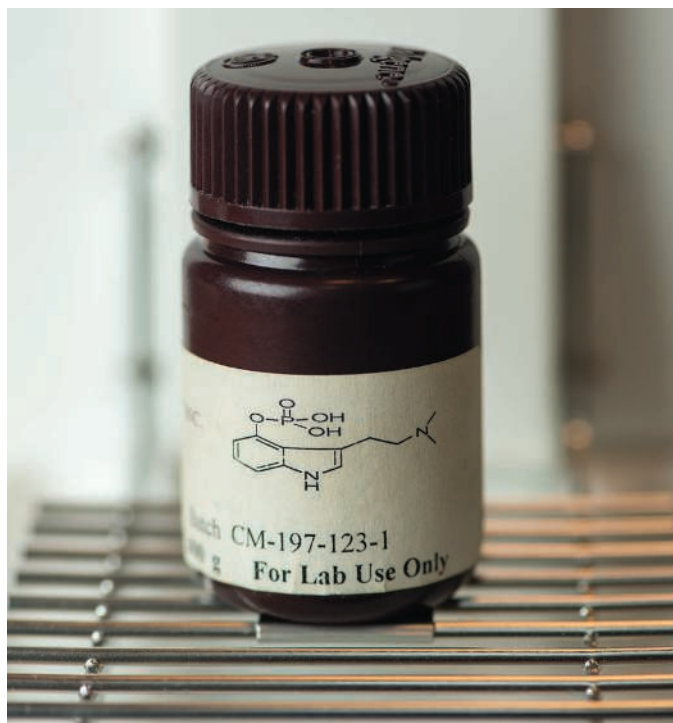
chedelic substances at Imperial College London. A 2012 meta-analysis, for instance, showed that in randomized, double-blind trials done between 1966 and 1970, 59% of alcoholics who received LSD showed lower levels of alcohol misuse in the months afterward, compared with only 38% of those who received placebo.

The experiments of that era went well beyond potential treatments. Some researchers sought to instill mystical or religious experiences in volunteers or help resocialize criminals. Others gave the drugs to artists and studied how it changed their work.

Hofmann, meanwhile, conjured a second demon from mysterious mushrooms said to have been used for sacred ceremonies in Mexico for centuries. Neither mice nor dogs showed any reaction to the mushrooms, so Hofmann again turned to self-experimenting. By testing

different extracts of the mushrooms on himself and co-workers and purifying those with a psychedelic effect, he managed in 1958 to isolate two equally active molecules, psilocybin and psilocin. Both are closely related to LSD, but psilocybin was the more stable one. Again, Sandoz began providing it to researchers worldwide.

IN A SMALL ROOM at a psychiatric hospital in Zurich, Martin is tripping. He shifts his position on the bed but feels, strangely, as if his body keeps moving. He sees a colorful mist swirling above the floor and the orange bedspread starts glowing. The student knows what that means: The five



A bottle of psilocybin in Ross's lab is weighed every day to prevent theft.

as a wicked witch, and he felt his sense of self dissolve. He thought he was losing his mind. "A demon had invaded me, had taken possession of my body, mind, and soul," he later wrote. The next morning, however, he woke up with a clear head, feeling euphoric. "I was aware that LSD, a new active compound with such properties, would have to be of use in pharmacology, in neurology, and especially in psychiatry," he wrote. Sandoz soon started producing the drug under the name Delysid and asked researchers all over the world to experiment with it.

Doctors and scientists embraced LSD. Some started giving patients single doses of up to 800 micrograms in hopes of in-



Harry Williams squirts LSD into the mouth of Carl Pfeiffer, a fellow physician, at Emory University in Atlanta in 1955.

unmarked capsules he was given 20 minutes ago contained not a placebo but rather 25 milligrams of psilocybin.

Martin is just one of hundreds of volunteers who have been sent on a psychedelic trip here over the years. The cradle of LSD and psilocybin, Switzerland is still a center for psychedelic research. Vollenweider started out in 1990, comparing the effect of LSD with that of ketamine and amphetamines for his Ph.D. thesis.

At the time, little was known about how psychedelics produce their extreme effects on the mind. Scientists had noticed, however, that their potency seemed to correlate with how strongly they bind to a class of serotonin receptors called 2A. In 1998, Vollenweider showed that blocking that receptor with the chemical ketanserin blocked psilocybin's effects. Most researchers now agree that classic psychedelics like LSD, mescaline, and DMT—the key component in ayahuasca, a psychedelic brew from the Amazon—work by engaging this receptor.

Just how that unleashes such overpowering effects is unclear, however. Vollenweider has published studies showing an increase in brain activity, particularly in the frontal cortex. Flooding that part of the brain with too much information may lead to hallucina-



In the 1960s, psychologist Timothy Leary studied how psychedelic drugs influenced the work of artists.

tions and fracture the mind, he says. But in a recent brain imaging study, Carhart-Harris found that psilocybin actually reduced activation in important parts of the brain. He thinks psychedelic drugs activate a particular set of neurons that disrupt the

regular pattern of activation in the cortex, the way a few irregular clappers can disrupt an audience clapping in synchrony.

The regular pattern is the basis of our sense of self, Carhart-Harris says: "When this organization collapses, the self dissolves." Regions that are usually controlled by the cortex become more autonomous, which may explain why people on psychedelics can have ecstatic experiences as well as terrifying trips. "The emotion centers have been let off the leash, if you want," Carhart-Harris says. Hallucinations may occur because the shift in brain activity seems to allow visual processing to be more easily influenced by someone's beliefs.

An hour after he has taken the capsules, Martin is lying in a scanner and feels as if he is floating. "All my negative feelings were erased. I kept grinning," he later recalls. On a screen, Martin is shown representations of himself and two other people playing a ball game together. After a while, the other two start excluding Martin from the game. In earlier studies, Vollenweider's group showed that people who take psilocybin respond less strongly to negative images, such as photos of an accident. Now, the researchers want to find out whether the drug can ease people's sadness about being excluded.

Vollenweider and Carhart-Harris both plan to give the drug to depressed patients. But they disagree on how it might help them. Vollenweider notes that an excess of serotonin 2A receptors in the frontal cortex is thought to contribute to depression; by binding to and overstimulating these receptors, psilocybin may trigger the brain to reduce their numbers, he says. On top of that, psilocybin may also trigger the release of a factor called BDNF, which

can help neurons make new connections.

Carhart-Harris, in contrast, thinks the antidepressant effect is related to the drug's ability to disrupt brain networks, in particular the so-called default network, which is active when people think about themselves.

Healing with a high?

A selection of recent and planned trials with psychedelic drugs

LOCATION	SUBSTANCE	INDICATION	TREATMENT	NUMBER OF PATIENTS	STATUS
Harbor-UCLA Medical Center, Los Angeles, California	Psilocybin	Existential anxiety related to cancer	Two experimental sessions a few weeks apart, one with psilocybin and one with placebo	12	Published in 2011
Private practice in Solothurn, Switzerland	LSD	Anxiety in patients with life-threatening disease	Psychotherapy, including two sessions on LSD	12	Published in 2011
Two independent clinics in Mexico	Ibogaine	Opiate addiction	Ibogaine-assisted psychotherapy	30	Completed in 2012; unpublished
New York University, New York City	Psilocybin	Existential anxiety related to cancer	Nine preparatory psychotherapy sessions; two dosing sessions (one psilocybin, one placebo)	32	Ongoing since 2009
Johns Hopkins University, Baltimore, Maryland	Psilocybin	Existential anxiety related to cancer	Several psychotherapy sessions, including one on psilocybin	44	Ongoing since 2009
Johns Hopkins University	Psilocybin	Nicotine addiction	Several preparatory sessions, then three daylong sessions with psilocybin	15 in an open-label pilot; 80 in a controlled trial against nicotine patch	Pilot completed; paper under review
Imperial College London	Psilocybin	Depression	Psychotherapy with two sessions on oral psilocybin	12 in an open-label pilot; 60 in a controlled trial	Expected to start by the end of 2014
University of Alabama, Birmingham	Psilocybin	Cocaine dependence	Several preparatory sessions, then one daylong session with psilocybin	40	Expected to start by the end of 2014

Source: ClinicalTrials.gov; participating researchers

Patients with depression spend too much time on self-reflection, Carhart-Harris says. “These people are stuck in their own heads, they cannot engage in the world.” Psilocybin may loosen that psychological spasm, he suggests. “It’s like taking a snow globe and shaking it.”

Florian Holsboer, director of the Max Planck Institute of Psychiatry in Munich, Germany, says both hypotheses are intriguing, but he is strongly opposed to treating patients with psychedelic drugs. “You can’t give patients some substance just because it has an antidepressant effect on top of many other effects. That’s too dangerous,” he says.

Holsboer says scientists should use psilocybin studies as a starting point to find similar substances that have antidepressant effects but aren’t psychedelic. Molecules that engage the serotonin 2A receptor but don’t cause a trip do exist, Vollenweider says; his group is planning to study some of them. But Carhart-Harris is convinced that for the treatment of depression, the psychedelic experience itself is essential. “This is not a chemical effect,” he says.

THE PSYCHEDELIC EXPERIENCE was the downfall of these compounds last time around, however, when enthusiasm for it got out of hand. To U.S. military and intel-

“I think psychedelic researchers should be neutral, bland, boring. I am not interested in destroying my career.”

Stephen Ross, Bellevue Hospital Center

ligence agencies, the new drugs seemed to offer exciting ways of fighting enemies both at home and abroad. In secret and sometimes highly unethical research, they tried to develop them into a truth serum and a psychochemical weapon that could disorient opposing armies. To millions of others, LSD and psilocybin seemed to offer the reverse: freedom and inner peace, escape from the material world, and heightened sexual pleasure. Harvard University psychologist Timothy Leary became the high priest of the cult, preaching a whole generation to “turn on, tune in and drop out.” Reports of drug parties at Leary’s home and allegations that he had given drugs to students led to his firing from the university.

As recreational use of psychedelic drugs spread in the United States—a trend that

never really caught on in Europe—their dark side started to show. The drugs are very safe in a research environment, where subjects are carefully screened, prepared, and supported by therapists, but they can have horrific consequences in other situations, Ross says. Psychedelics can induce psychoses and make people agitated and violent toward themselves or others; people at risk of psychoses should never use them. In the ’60s, more and more users ended up in emergency rooms, and newspapers ran stories about people walking in front of a car or jumping out of a window because they believed they could fly.

LSD became a liability for its maker. In a letter in August 1965, Sandoz announced it would stop shipping the drug because its danger “has increased considerably and in some parts of the world has reached the scale of a serious threat to public health.” In 1970, former President Richard Nixon signed the Controlled Substances Act, which classified drugs into five schedules according to their dangers and medical usefulness; LSD, psilocybin, and other hallucinogenics were placed in schedule I. A year later, the United Nations adopted the Convention on Psychotropic Substances. Many countries adopted laws similar to those in the United States.

In theory, the regulations allowed for scientific investigation of the substances, but there were many hurdles and funding was drying up. Moreover, the drugs had developed such a bad reputation that scientists on both sides of the Atlantic lost interest, Griffiths says. "The whole system shut down."

WHEN TAMMY BURGESS WAS diagnosed with ovarian cancer, the tumor had already metastasized. A CT scan of her abdominal cavity showed many little growths. "It looked like oatmeal," the 55-year-old New Yorker says. The cancer had also spread to her lungs. Paralyzed, Burgess laid on her couch for hours, watching old episodes of *The West Wing*. "This overwhelming sense of dread just permeates every cell of your

body. You think about being ill, about not wanting to die," she recalls.

Burgess had 9 weeks of chemotherapy, then a surgery—she calls it the decancering—then more chemotherapy. The treatment was very successful, and Burgess has had no recurrence. But as it neared its end, she got more and more depressed. She couldn't sleep, couldn't stand silence. The image of the cancer on an ultrasound stuck in her mind: "All these little tiny circles, seeing that menace floating inside of me, just gray and black, silent and eerie," she says. "I don't think I ever felt the kind of relief that my friends and relatives were feeling."

Burgess enrolled in Ross's study of psilocybin. An addiction specialist, Ross had become interested in psychedelics in 2006. "In

psychiatry training, I had learned that these substances were banned, dangerous, and addictive. I didn't learn anything about their history and all the research that took place from the '50s to the early '70s," he says. To him, "the therapeutic application of mystical, spiritual states within psychiatry" was a completely new idea.

Ross and two colleagues formed a study group to educate themselves. They learned that a doctor named Rick Strassman at the University of New Mexico, Albuquerque, had scraped together funds for a DMT study in 1990. David Nichols, a pharmacologist at Purdue University in West Lafayette, Indiana, who had produced the DMT for it, had founded a nonprofit called the Heffter Research Institute in Santa Fe a few years

Can ecstasy treat the agony of PTSD?

by Kai Kupferschmidt

Rick Doblin was 29 when he first tried 3,4-methylenedioxymethamphetamine (MDMA). The experience was life-changing, Doblin recalls 30 years later. At the time, some psychotherapists had started giving the drug to patients, claiming it helped them talk and fostered a more trusting atmosphere. Doblin—who took the drug privately—experienced all that and more; MDMA "helped open me up to my emotions and enabled me to feel deep and profound love," he says. He decided to study clinical psychology and devote his career to giving MDMA a role in therapy.

Today, MDMA has become known primarily as the illegal party drug ecstasy—but Doblin has kept his plan alive. The Multidisciplinary Association for Psychedelic Studies (MAPS) in Santa Cruz, California, which he leads, has funneled millions into studies of MDMA as a treatment for post-traumatic stress disorder (PTSD), despite political headwinds and scientific skepticism. Two phase II studies have been completed, one with encouraging results, and several more are in progress.

Although often lumped together with psychedelics like LSD or psilocybin, MDMA has a different mode of action and doesn't produce hallucinations. It activates brain receptors for dopamine and noradrenaline and releases serotonin from nerve endings, leading to the characteristic feeling of euphoria that made it popular in

clubs and at dance events.

Its potential to help PTSD patients is clear, says psychiatrist David Nutt of Imperial College London. Such patients relive a psychological trauma again and again and are often plagued by fear and thoughts of suicide; therapists try to help them master their memory by revisiting it in a safe atmosphere without overwhelming emotions. MDMA might make that easier because it reduces fear and fosters trust, Nutt says.

But questions about safety have long dogged MDMA. Some clubbers using it died from dehydration, and there have been reports of brain damage; in 1985, the U.S. Drug Enforcement Agency (DEA) made MDMA illegal and ranked it among the most dangerous drugs. Many other countries followed suit. This kept MDMA out of psychotherapeutic practice but didn't do much to stop its use elsewhere; almost 20 million people consumed the drug in 2011, according to United Nations estimates. And some scientists say the risks have been overblown. Nutt, for one, argues that using MDMA is safer than horse riding (*Science*, 31 January, p. 478).

Following DEA's decision, Doblin changed career plans and studied public policy—and in 1986, he founded MAPS to pursue his dream. In 1992, the U.S. Food and Drug Administration (FDA) allowed a phase I safety study, which Doblin and psychiatrist Charles Grob of the University of California, Los Angeles, conducted from 1993 to 1995. They then started to prepare a study to treat cancer



patients' anxiety with MDMA, but in 1999, Grob decided to focus on psilocybin instead. FDA approved an MDMA study in PTSD patients in 2001, and it took 3 more years to get the green light from institutional ethics panels and DEA.

The study, finally published by Doblin and South Carolina psychiatrist Michael Mithoefer in 2010, enrolled 20 patients, most of them female sexual assault victims. After 2 months, 10 out of 12 patients who had received MDMA along with psychotherapy no longer met the diagnostic criteria for PTSD, compared with only two out of eight patients who had received a placebo.

It was a small study, and another trial in Switzerland showed no significant effect. But MAPS is now funding further phase II studies in Israel, Canada, and the United States. The association, which has also funded some work on classic psychedelics, employs 14 people and took in more than \$1.5 million in private

later to support research into psychedelics. At about the same time, the Multidisciplinary Association for Psychedelic Studies had started a safety study of MDMA, better known as ecstasy (see sidebar). Ross and his colleagues learned that these events had kicked off a small research renaissance, which they joined.

Leary's legacy lingers in the minds of modern-day researchers and regulators, however. Psychedelics researchers tend to avoid discussions about their own experiences. "I think psychedelic researchers should be neutral, bland, boring," Ross says. "I am not interested in destroying my career." Privately, many admit having tried the drugs. Vollenweider, who has taken psilocybin as part of a dosing study, says it is hard

to understand the drug's effects without firsthand experience. And patients may feel safer if therapists know the unusual terrain themselves, Ross says.

The reemerging field also struggles with some unresolved methodological problems. Many of the new studies are small, and the profound effects of psychedelics "make it virtually impossible to blind subjects," says John Kelly, an addiction psychiatrist at Massachusetts General Hospital in Boston.

And there are other hurdles. Ross needed a license from the Drug Enforcement Administration, and he had to install a safe. "The security around it is a little bit ridiculous," he says. Bellevue Hospital Center, where Ross works, is a city hospital, and some administrators feared that the scientists could be accused of testing psychedelic drugs on poor, dying minority patients. "There were many times that the project was almost finished," Ross says. Eventually, the dental school offered to host the study. A room was decorated to make patients comfortable. It has a sofa bed and black-and-white photographs of paths snaking into the woods or toward the horizon. Small mushroom sculptures and a copy of the *The Tibetan Book of Living and Dying* make it feel like the comfy bedroom of a hippie aunt.

ONE FRIDAY MORNING, Burgess came into that room carrying a few personal things: a blanket, a picture of the house she grew up in. The therapists, a man and a woman, explained what would happen once more and went over the safety aspects. An antipsychotic medicine was at hand, Burgess was told, in case she wanted to end the experience. Then, she was handed a big chalice with the psilocybin capsule inside. She swallowed it with some water, lay down on the couch, and put on headphones and eyeshades.

Soon, Burgess started to float in a black space, surrounded by beautiful bright stars in various colors. "I kept hearing 'Hi. Hi,'" she says. She heard herself saying "Hi. I'm Tammy from Earth ... " Later, ancient stone faces appeared, crumbling to dust then taking shape again. She met a doctor and asked if there would ever be a cure for cancer. "It doesn't matter," he answered. "Everyone has to die." That comforted her in a weird way.

By the time Burgess went home that night, she felt like something had lifted. At work, someone told her that she seemed her old self again. "I just got better," she says. Ross says he has seen many similar experiences. "The first couple of years, I felt like I was in the wilderness; I was hanging out with a bunch of old dudes that had kept the flame flickering all these years but were tired," Ross says. "I thought to myself, 'This is the



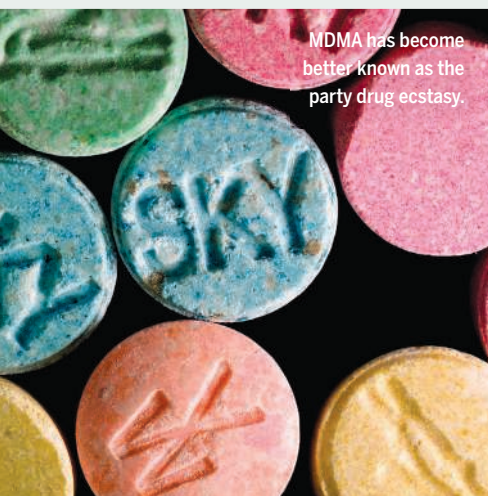
Psilocybe semilanceata, also known as the liberty cap, is one of many mushroom species that contains psilocybin.

stupidest thing I have ever done.' " Now, he's convinced he's on to something.

The study is scheduled to finish later this year and Ross hopes to publish results soon afterward. "We have taken a peek at the data and there seems to be a very big treatment effect of psilocybin against placebo," he says. A similar phase II study is going on at Johns Hopkins; Ross is already thinking about a phase III trial that might lead to Food and Drug Administration approval.

But Vollenweider is critical of Ross's approach. He says it's based on the idea that the more a patient's self dissolves, the more mystical their experience is, the better. "For me that is not a medical concept. It is more like an interesting shamanic concept," he says. Others have argued that the overwhelming sense of meaningfulness that Burgess and other patients feel is just another of the drug's illusions. In his essay *Return Trip to Nirvana*, the writer Arthur Koestler describes listening to music after taking psilocybin. "I suddenly understood the very essence of music, the secret of its magic," he wrote. The next day, he couldn't even say what kind of music it had been. "I may just as well have listened to Liberace. ... [M]y soul was steeped in cosmic schmalz."

But to Burgess, the experience was real enough. "I believe that when I pass, this is what I will experience again," she says. And Nichols says the debate about the true meaning of trips is irrelevant for most patients. "If it gives them peace, if it helps people to die peacefully with their friends and their family at their side," he says, "I don't care if it's real or an illusion." ■



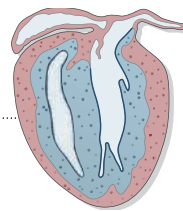
MDMA has become better known as the party drug ecstasy.

donations in fiscal year 2013 alone.

Neurobiologist Franz Vollenweider of the University of Zurich in Switzerland calls the MDMA results "exciting," but is critical of MAPS. "They annoy me, because they pretend to know all the results before a single study is done," he says. "It's a political organization" that hopes to decriminalize the drug, he adds. Vollenweider calls Doblin "a dreamer" who won't be able to finance or manage a phase III trial.

Doblin concedes that he thinks the ban on MDMA should go. "I believe in people's basic human right to use molecules to explore their consciousness," he says. But he's confident he can raise the \$15 million needed for a phase III study in 400 patients. MDMA could become a prescription medicine by 2021, he predicts. "I might just get to start my psychedelic psychotherapist career shortly before I turn 70." ■

PHOTOS: (TOP TO BOTTOM) ALAN ROCKEFELLER; DEA



LETTERS

Edited by Jennifer Sills

Science ethics: Young scientists speak

What is the most challenging ethical question facing young investigators in your field? How should it be addressed? In April, we asked young scientists to tell us their thoughts. A sample of their responses can be found below. To allow for as many voices

NEXTGEN VOICES

as possible, in some cases we

have printed excerpts of longer submissions (indicated by ellipses) and lightly copyedited original text for clarity. To read the complete versions, as well as many more, go to <http://scim.ag/NextGen11Results>. Follow *Science's* NextGen VOICES survey on Twitter with the hashtag #NextGenSci.



MEDIA COMMUNICATION about stem cell research, nanotechnology, and other novel medical biotechnologies often fuels public expectations for imminent health applications. The pace of science, however, is often incremental and falls short of hopes.

Information that promotes optimism is necessary to attain public support and mobilize science, but poorly informed hope leads to disappointment, despair, and distrust. Compounding these challenges is the impact that hype can have on policy agendas, with a premature focus on translation at the cost of basic science. A key ethical question, therefore, is: How ought we communicate about the promise of novel biotechnologies with the aim of catalyzing public support while avoiding hype? Social responsibility in science communication is the answer. Media should strive for accurate reporting, accounting for the scientific merits and caveats of research. Likewise, scientists communicating with the media about their research should highlight both roadblocks and progress.



Educational approaches must be extended, particularly targeting scientists-in-training as future spokespersons. Clinicians could address media content in their clinical practice to help patients—the end-users of biotechnologies—develop informed hope. Whereas a body of research predominantly focuses on managing the content of science communication in the public sphere, future research should aim to characterize the influence of promotional communications on patient decision-making. Balanced science communication will encourage support for scientific progress and ground expectations in the limits of science and current clinical tools.

Shelly Benjamy

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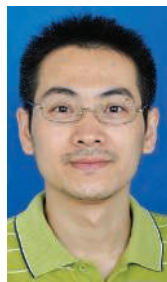


...WHEN SCIENTISTS fabricate their data, eventually they get caught. But because the timeline for detecting fraud is slow, the skewed data may in the meantime already have been used as a foundation for the latest “breakthrough.” Why

not catch the criminals before they commit the crime? Scientific journals do have strict review processes in place, but these processes are not rigorous enough. Data modifications, regardless of size or type, can easily be overlooked. I propose that the best journals in the world require proof of data replication by another researcher before submission for publication. The social and financial stigma against research that strictly confirms the results of others should be replaced by healthy encouragement and funding of outside validation as a way to provide checks and balances in the scientific community.

Rosalie Doerksen

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...THE MOST CHALLENGING ethical question facing young investigators is a lack of scientific integrity, which may damage the reputation of both young individuals involved and the whole scientific enterprise, and dampen the ability of young scientists to produce original innovations. To

address this problem, promoting education

in responsible conduct of research is the first important step in China, where most universities and institutes did not offer official courses concerning scientific integrity or a code of scientific conduct until recently.... Another deserving effort in promoting scientific integrity in China is to foster a healthy research environment, which includes making explicit research ethics policies, issuing a practical code of conduct, establishing a credible and authoritative national organization to supervise local units, protecting whistleblowers, building a rigorous and fair peer review system, revising the criteria for promotion and reward to emphasize research quality rather than quantity by a researcher, and achieving zero tolerance for unethical research behaviors.

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THE USE OF animals in scientific research is the most controversial, divisive, and ethically challenging question I face. That innocently seeking question, “...and what do you do?” has so many times led to a long and heated debate on whether animal research

is ethical or necessary. The majority of our medical and scientific achievements are developed, tested, or discovered with the help of the ubiquitous lab animal. This is a nice sentiment, but it often misses the mark when defending animal research, and who is really surprised? As far as the public are concerned, our research is just inaccessible and incomprehensible jargon. The very animal scientific procedure act (UK) we work under contains a secrecy clause, published articles are hidden behind online paywalls (even those paid for by the taxpayer), and access to animal laboratories is restricted. Ultimately, shutting out the public like this is inimical and will eventually exaggerate the public's view of scientists as cryptic, egotistical, and cold. Through constant public engagement, social communication, and early education, people could have a much better understanding of why the laboratory animal is indispensable. Such projects as the recent Declaration of Openness on Animal Research Concordat, the Elsevier “cost of knowledge” boycott, and the petitions to have our secrecy clause lifted are all in favor of educating the public. It is imperative that as scientists we seek to support

such causes wherever possible, if not for the benefit of the public, then for the benefit of science in the long run.

Roddy Grieves

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IN GENETICS RESEARCH, I am expected to work with high-throughput sequencing, which regularly collects and processes data spanning entire genomes. With so much genomic data and computational power at hand, it is increasingly likely

I will encounter incidental findings of BRCA, CF, Alzheimer's, or other flagged genes. However, with samples coming from distant sources, the question arises of my ethical responsibility to contact the sample provider and make sure that the donor is informed. Having donors fill out consent forms asking if they would like to be notified in the case of relevant findings and then subsequently marking biological samples would solve the dilemma that investigators like myself face when dealing with sensitive genomic data.

Girish Valluru

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...MEDICINE IS INCREASINGLY becoming too complicated for many individuals to understand. It is beginning to surpass the ability of patients to make informed decisions, especially around new emerging biotechnologies such as stem cell

interventions and genome sequencing. The burden is increasing on our new generation of young investigators to disseminate increasingly complex science to the community; however, there is no formal training offered in this endeavor. We need to educate new scientists and clinicians about these aspects of knowledge translation, as well as the public about medical tests and procedures, so that patients can truly become empowered to make informed decisions.

Karen J. Jacob

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AN ETHICAL ISSUE facing young investigators in neurosciences pertains to the use of cognitive-enhancing interventions in healthy individuals. ...A notable example on university campuses is the use of ADHD drugs such as Adderall and Ritalin by

healthy students to improve performance on exams and work efficiency. Some argue that we should welcome new methods of improving our cognitive function and that the use of neurocognitive enhancing drugs is essentially no different from increasing our work efficiency through the use of caffeine. On the other hand, the use of usually illegally purchased and not prescribed drugs may have detrimental effects on the user; the long-term side effects of ADHD interventions on healthy individuals have not been extensively studied. Also the ethical issue presents itself as to whether it is fair in an academic setting for those who take cognitive-enhancing drugs to be assessed against those who do not take such drugs, especially considering the increasing competitiveness for admission to graduate programs. I suggest that we approach this issue first by educating students on the possible dangers of not prescribed medications and second by investing more research to truly understand the impacts of cognitive enhancing drugs on students competing in an increasingly demanding academic environment.

Cody Lo

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LARGE-SCALE, MULTI-center clinical trials are the way of the future for young investigators wishing to address complex questions related to brain development and to discover safe and effective interventions for kids living with neurodevelopmental

disability. ...However, important ethical challenges must be addressed before the power of Big Data can be harnessed to its fullest potential. For instance, how do we achieve consensus among researchers, pharmaceutical industry representatives, regulatory agencies, research ethics boards, and family advocates on best ethical practices for conducting large-scale multicenter clinical trials involving

children and adolescents living with neurodevelopmental disability? What mechanisms need to be in place to ensure ethics harmonization and oversight? Work is currently being done to achieve this at both national and international levels. Young investigators need to be engaged in the harmonization process, as they are on the front lines of data collection and analysis and are uniquely positioned to interface with actors on many levels: senior researchers, research staff, ethics boards, and the research participants themselves.

Nina Di Pietro

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ENVIRONMENTAL research requires money and logistical support, particularly if the science being conducted is cutting-edge and uses the latest technologies. Obtaining this funding can be difficult—even as government science budgets increase, the

proportion of money available for research that will not produce direct financial benefits has waned. Therefore, young researchers who tackle important environmental questions may find themselves in a position of tailoring their programs to involve industry partners that can support their work. How can these young investigators accept money and in-kind support from an industry partner while ensuring that they maintain a real and perceived ability to conduct and publish unbiased science? There are tremendous positive opportunities in forming research partnerships, but there are also risks, particularly of a real or perceived conflict of interest. Researchers should be trained in how to enter into these partnerships in such a way that the integrity of their research program is not compromised. In addition, the scientific community should establish transparent best-practice guidelines that can be followed to ensure scientific impartiality, so that it can be clearly identified when research is produced through a defensible funding arrangement. Audits should then be conducted to identify whether researchers and partners are following these rules.

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THE MOST CHALLENGING ethical question facing aerospace engineers is the protection of the space environment. Although young scientists and engineers contribute increasingly to the space exploration and development, we

have not given adequate attention to the possible connections between the advanced technique and environmental consciousness. In my opinion, we ought to broaden access to environmental philosophy and realize that unbridled space activities by manned and robotic missions may pollute, degrade, and even destroy the space environment. In addition, young investigators should be encouraged to participate in the discussion of ethical code and policy for future space program. The seniors can help us develop the conception of a sustainable and environmentally aware exploration of the space environment for industry, commerce, and tourism.

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ALTHOUGH THEY ARE sufficiently warned against committing outright fraud through ubiquitous ethics seminars, young investigators are still exposed to a more subtle, lurking enemy challenging their professional integrity: scientific pollution. This

murky underworld of junk science includes everything from improper citations to perpetuation of invalidated ideas to gross misinterpretation of data. Young investigators are particularly prone to (often unknowingly) muddy the scientific waters because as they learn the lay of the land, they take cues from other papers, peers, and mentors. So when a graduate student reads a review paper citing other review articles instead of original work, this becomes their standard of what is acceptable; never mind that if you trace the cited fact to the original study, you may find a 1965 publication using an outdated method now known to be unreliable. A postdoc designing a new experiment may opt to use currently available methods—even if they are far from the best—for the sake of feasibility or consistency with prior work, unwilling to question their mentor's established, affordable methods. Junior investigators

struggling for grants and promotions may be tempted to overstate their findings or publish premature findings in lower-tier journals to boost their publication numbers. Because all this nonsense makes good science more difficult for everyone, it is our collective responsibility as scientists, colleagues, communicators, advocates, and mentors to curtail it wherever possible. Research thoughtfully. Analyze critically. Publish scrupulously. Speak truthfully.

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THE PRESSURE TO publish can lure young people to fake data, ignore data, or jump to conclusions from an insufficient number of data. ...The “publish or perish” game is bad for science and for the scientist. Instead of quantity, we should go for quality.

I strongly support an idea of my professor in which each scientist starts with a limited number of publication slots, and he/she is only allowed to publish more if the previous publications are deemed good enough.

Ádám Kun

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PRIVACY AND DATA collection have become the most challenging ethical issue in telecommunication and wireless systems. Now that coherent optical technology is used to deliver 100 gigabit through fibers, and microprocessors and permanent memory are

made inexpensive, how do we guard the massive amounts of personal data submitted to commercial entities when ordering online or using mobile apps? Imagine a world where unique fingerprints, facial dimensions, and palm patterns unknowingly become available to physicians, insurance companies, and research entities, but are not protected by privacy or anonymity laws, while unencrypted patient data could be lost or stolen at any minute.... Soon, cellphones and GPS will know your route everywhere, with speeding tickets coming directly from private industry, not

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Thanks to Kefeng Li at the Department of Medicine, University of California, San Diego, for submitting this question.

Deadline for submissions is 15 August. A selection of the best responses will be published in the 3 October issue of *Science*. Submissions should be 250 words or less. Anonymous submissions will not be considered. Please submit only once.

the police. ...It's time for us to think about what kinds of information should be collected and retained. The gap between data collection and privacy protection needs to be bridged through appropriate policy discussions. And the principles of purpose limitation and data minimization need to be addressed at the soonest possible time to balance the benefits for businesses and researchers against God-given constitutionally protected individual privacy rights.

Yi Weng

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BUREAUCRACY AND guanxi (cultivating relationships with people who can help you) form a major challenge to science ethics in China. Most young investigators have to bear great pressure and be kept busy in the bottom of the pyramid. Only a few can do

something of personal interest. Boss professors are busy participating in conferences, developing guanxi, and grabbing projects and funds, while young investigators have to practically fulfill these projects and write papers with the names of the boss professors in the author list, sometimes as the first author, even if the boss professors do not know what was written in the paper. There is a circle of guanxi, and the most successful young investigators in China are often the former students of famous professors who hold or formerly held critical positions as academic bureaucrats or referees in judging projects and talents. ...If the younger generation of Chinese investigators wants to achieve better development in China, they must comply with famous professors and develop guanxi, or obtain a Ph.D. degree

and work experience abroad and then become awarded returnees.... China needs reforming mechanisms, institutions, and laws to remove interference from bureaucracy and guanxi in allocating research resources....

Xin Miao

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MY FIELD IS COMPUTER security. I use techniques aimed at detecting malicious attacks on computer systems, and I need to operate in the twilight zone between good and bad. Research on new intrusion detection algorithms is often at best questionable

and at worst invalid, given that the results cannot be verified under realistic conditions. Testing under realistic scenarios is problematic, because the results are not repeatable. Furthermore, testing on real data requires high-security vetting and may produce confidential results that are not publishable. Security technologies may also have dual-use possibilities. Security researchers may, for example, detect new system vulnerabilities during their research, and may end up in ethical dilemmas: Shall I be good and publish these results or shall I sell them at the highest price to the gray market of government agencies and others that capitalize on vulnerabilities? And then you have privacy: These technologies can be used both for the good—protecting security of citizens—and bad—creating an Orwellian surveillance society. ■

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DEVELOPMENT

A crowning achievement for deciphering coronary origins

Postnatal maturation of the heart spurs coronary vessel development from an unanticipated source

By C. Geoffrey Burns^{1,2,3} and
Caroline E. Burns^{1,2,3}

Named for its crownlike appearance (1), the coronary circulation comprises arteries and veins that transport blood to and from the heart's muscle, respectively. When a large coronary artery becomes obstructed, a downstream segment of cardiac muscle dies from oxygen deprivation in a pathologic condition called acute myocardial infarction (2), or heart attack. Over time, the injured heart replaces damaged muscle with scar tissue (3) that maintains cardiac wall integrity but also adversely affects pump function, which often leads to heart failure (4). Therapeutic interventions that stimulate the growth of new cardiac muscle to supplant scar formation would substantially improve heart attack outcomes (5). The success of any such strategy would also rely on concomitant growth of new coronary blood vessels. Therefore, a comprehensive understanding of how this specialized vascular bed develops is a prerequisite for regenerative strategies designed to reduce the morbidity and mortality caused by heart failure after myocardial infarction. On page 90 in this issue, Tian *et al.* (6) demonstrate that a large fraction of coronary vessels in the mouse heart form during postnatal life through a unique mechanism.

Formation and maturation of mammalian heart muscle (myocardium) is a dynamic process that begins during embryogenesis and continues after birth (6, 7). The primitive embryonic heart is composed of a compact layer of muscle lined internally by specialized endothelial cells, the endocardium. At this stage, the walls of the embryonic heart provide a smooth surface for blood flow. As heart development proceeds, a second layer of cardiac muscle, with meshlike tissue architecture, emerges between the compact muscle layer and endocardium (see the figure). This trabecular layer comprises a collection of thin, interconnected strands of muscle (trabeculae)

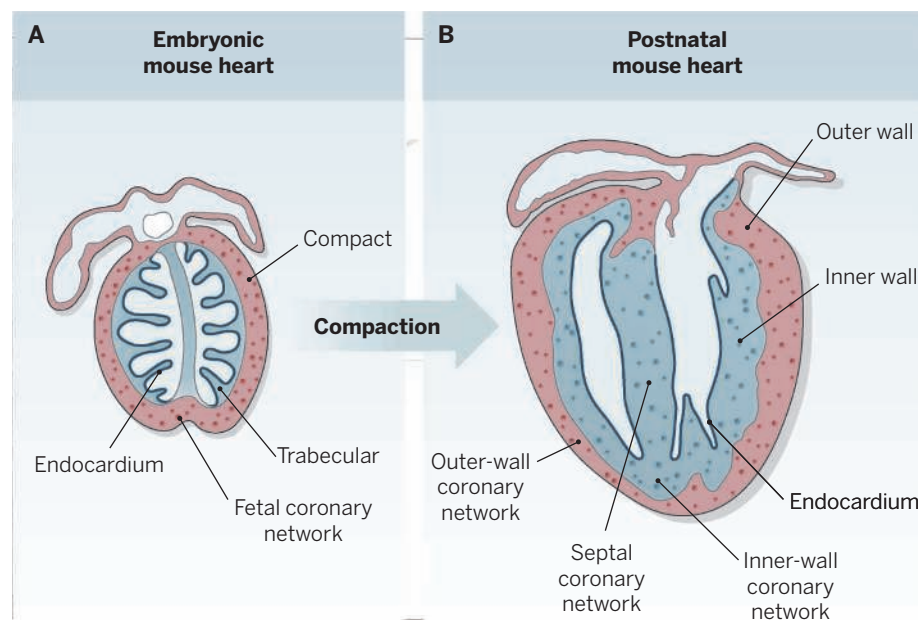
derived from, and anchored to, the compact layer. Beginning prior to birth and ending in the postnatal period (6, 7), the trabecular layer of myocardium undergoes "compaction," in which its meshlike muscle densifies onto the compact layer, causing the latter to thicken. As a result, the adult myocardial wall contains an outer segment derived from embryonic compact muscle and an inner segment that forms through trabecular compaction.

The two embryonic myocardial layers have differential requirements for a coronary circulation. The compact myocardium receives oxygen from a fetal coronary network (8–12). By contrast, the trabecular layer receives oxygen by diffusion from chamber blood (13). As compaction of trabecular muscle ensues, new coronary vessels emerge in this region. Until this study by Tian *et al.*, the inner-wall coronary vessels were thought to arise by angiogenic invasion of the fetal coronary network (7). However, upon lineage tracing the endothelium of

this fetal coronary system, Tian *et al.* discovered that inner-wall coronary vessels remained unlabeled, thereby highlighting an alternative source.

Tian *et al.* turned their attention to the endocardium. In addition to its close association with trabecular muscle, the endocardium decreases in surface area as the result of compaction, thereby creating excess endothelial cells potentially available for repurposing. The authors generated a mouse strain to exclusively label endocardial cells prior to coronary vessel formation. In the mature heart, a majority of inner wall vessels were labeled by the lineage trace, demonstrating that a subset of endocardial cells originally functioning as the endothelial lining of the trabeculae become repurposed for transporting blood to heart muscle. This mechanism for generating coronary vessels in the inner wall also establishes the circulation of the muscular wall separating the right and left sides of the heart (interventricular septum) that forms prior to birth also by compaction (6).

Tian *et al.* provide evidence that coalescence of trabecular myocardium traps endocardial cells, causing them to break through their basement membrane prior to forming a vascular plexus that matures into the inner-wall vasculature. When compared to the fetal coronary lineage trace, the endocardial trace was a near perfect mirror image, demonstrating that the mature coronary circulation is constructed



Heart vessel development. (A) The compact layer of the embryonic heart is irrigated by a fetal coronary circulation. Meshlike muscle (the trabecular layer), lined by specialized endothelial cells (endocardium), develops on the chamber walls. (B) During the perinatal period, the trabecular muscle condenses and traps a subset of endocardial cells that seed formation of a substantial fraction of cardiac vessels. This observation contradicts the prevailing notion that sprouting of the fetal coronary network generates all of the heart's vessels.

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de novo from two mutually exclusive sources. In the adult mouse, 60% of the heart muscle is irrigated by vessels formed from endocardial cells during trabecular muscle compaction. Tian *et al.* speculate that delayed de novo creation of such a large proportion of the coronary network serves to meet the rapidly increasing metabolic demands of the thickening myocardial wall that would otherwise be unattainable by angiogenic expansion of the fetal coronary circulation.

Future studies will be required to delineate the molecular mechanisms that regulate postnatal development of the inner-wall vasculature and how they compare with those regulating establishment of the fetal coronaries. Tian *et al.* provide preliminary evidence that the former involves hypoxia and vascular endothelial growth factor, both implicated in fetal coronary development (12). An issue that remains to be resolved is the degree of interdependence between trabecular compaction and inner-wall vessel formation. If compaction is the driving force for vessel formation, then mutant mice that fail to undergo compaction (7) should lack inner-wall vessels. Alternatively, inner-wall vessel assembly might actively stimulate compaction. In this regard, it would be informative to analyze compaction in a mouse incapable of the endocardial to coronary endothelial lineage conversion. Another open question is whether this lineage conversion involves dedifferentiation to an angioblast state. It will also be interesting to identify the source of additional cell types, such as smooth muscle, presumed to associate with inner-wall coronary vessels. Ultimately, a complete understanding of this process will inform the development of therapeutic strategies to stimulate regeneration of coronary vessels from the endocardium of an infarcted heart. ■

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MICROBIOLOGY

The birth of cooperation

Mutualistic symbiosis can arise without prior coevolution

By Duur K. Aanen¹ and Ton Bisseling^{2,3}

Mutually beneficial associations between individuals of different species, called mutualistic symbioses, have enabled major ecological innovations and underlie some of the major transitions in evolution (1). For example, the ancestor of plants domesticated endosymbiotic photosynthetic bacteria, today's chloroplasts, for carbon fixation. This association dramatically increased the habitat of these photosynthetic bacteria from the sea to terrestrial ecosystems. However, the colonization of land by plants required an additional symbiotic association, with fungal root symbionts that facilitate nutrient uptake (2). Yet, surprisingly little is known about how mutualistic symbioses evolved and persist. On page 94 of this issue, Hom and Murray show how mutualism may arise without prior coevolution (see the photo) (3).

For a mutualistic association to form between different species, the costs of the interaction must be outweighed by the benefits individuals receive from their partner symbiont (4). However, how this condition can be fulfilled has remained enigmatic. According to one model, mutualism follows coevolution (see the figure, panels A, B, and C), for example, after a start as parasitic interactions (5) or commensalism (6). Under certain conditions, for example, if the reproductive interests between the two partners become united via joint reproduction, several coevolutionary responses may favor mutualistic interactions (1, 7, 8). However, Janzen has argued that an organism may appear to have coevolved with a different organism but is in fact just using the ecological opportunity, a hypothesis known as ecological fitting (see the figure, panel D) (9).

Hom and Murray now provide experimental evidence for ecological fitting. They first tested whether a mutualistic interaction between two species—the yeast *Saccharomyces cerevisiae* and the alga *Chlamydomonas reinhardtii*—could be established without introducing any genetic

modifications. The authors created an ecological setting in which the species can only survive when they establish a reciprocal carbon (C) and nitrogen (N) exchange. The yeast, but not the alga, can use glucose as a C source, whereas the alga can use the waste product of glucose metabolism, carbon dioxide (CO₂), in photosynthesis. On the other hand, the alga, but not the yeast, can use nitrite as a N source, but the yeast can use the conversion product of nitrite



Mutualism in the lab. *Chlamydomonas reinhardtii* grown alone (left) or in coculture with a nitrite-deficient mutant of *Neurospora crassa* (middle and right, two replicates). For further details, see (3).

metabolism, ammonia (NH₃). When the two species are cocultured with glucose as the sole C source and nitrite as the sole N source, the alga depends on CO₂ provided by the yeast and the yeast on NH₄ produced by the alga.

Hom and Murray show that under these conditions, the two species become completely dependent on each other: They become obligate mutualists. Surprisingly, the morphology of the algal symbionts differs from that of their free-living forms. This was particularly obvious in the interaction with the filamentous fungi *Neurospora crassa* and *Aspergillus nidulans* (relatives of lichenous fungi). Hom and Murray show that these fungi can also form an obligate mutualism with *C. reinhardtii*, but only if they have lost the ability to reduce nitrite via genetic engineering. The interaction of these filamentous fungi with *Chlamy-*

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domonas resulted in a close association, in which hyphae and algae formed a “beads on a string”-like organization. This close association is most likely a prerequisite for the mutualistic interaction, given that agitation markedly reduced the efficiency of the mutualism. The cell wall of *Chlamydomonas* changed markedly when associated with fungi.

The authors also tested whether mutualistic symbiosis could be established if only one partner depends on the association. This was the case when yeast depended on the interaction and the alga did not, but not vice versa. This finding can be understood by the difference in generation time between the two organisms: *S. cerevisiae* has a markedly shorter generation time than *C. reinhardtii*, and the faster-growing species can outcompete its partner if it is not dependent on it. These results suggest that, in a mutualistic interaction, where one partner cannot control the other, the slowest growing partner needs to be indispensable for a mutualism to get started.

Lichens, the symbiotic association superficially most similar to the engineered *C. reinhardtii*/*S. cerevisiae* mutualism, seem to be in conflict with the hypothesis that the slowest-growing partner needs to be indispensable for the establishment of a mutualistic symbiosis. Lichens consist of a filamentous fungus and photosynthetic green algae (or cyanobacteria) that exchange nutrients. In most lichen species, the fungus is obligatorily dependent on the association with algae, whereas the faster replicating algae still have independent life stages (10). However, in contrast to the mutualistic symbiosis created by Hom and Murray, the partners in lichens greatly differ in power: The fungus is the dominant partner and functions as a host species for the algal symbionts, with fungal tissue enclosing the photosynthetic partner (11). In general, in most mutualistic symbioses, one partner (the host) controls the reproduction of the other partner (the symbionts), usually in enclosed compartments. For these host-symbiont interactions, generation time of partner species has become largely irrelevant.

Extant symbioses tend to have a history of coevolution, potentially obscuring their origin (see the figure). But even if there is a

dominant host and a submissive symbiont in most extant symbioses, the partners may initially have been equivalent in power. For example, for lichens, a scenario of ecological fitting followed by coevolution seems plausible (see the figure, panel D). However, the specialized nature of other symbiotic interactions, such as those between rhizobia bacteria and nitrogen-fixing plants, makes an origin via ecological fitting highly unlikely. Rhizobia are hosted within specialized cells of root nodules. The formation of these organs on legume roots is induced by specific signal molecules, Nod factors, secreted by rhizobia. The genes required for Nod-factor production have no other function than for symbiosis, which strongly suggests that acquisition of these genes was an evolutionary change prior to mutualism (see the figure, panel C). *Parasponia* is the only genus outside the legume family that also can establish a nodule symbiosis (12). It has a recent evolutionary origin as the closely related sister genus, *Trema*, is non-nodulating. Because rhizobia are unable to induce any symbiotic response in *Trema*, it is very likely that genetic changes occurred in the host before nodule symbiosis (see the figure, panel C). Whether the complex nodule symbiosis is preceded by a simpler en-

dophytic interaction can now be addressed, because in certain habitats both plant species are present.

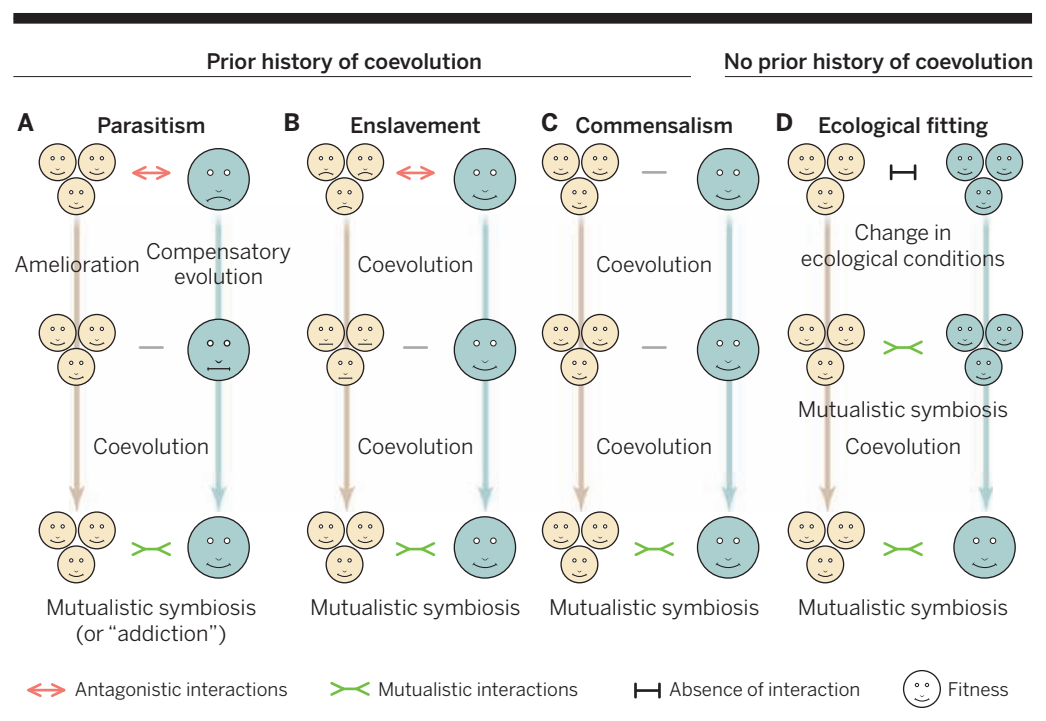
As Hom and Murray show, mutualistic symbioses with seemingly derived morphological changes can arise spontaneously. The question that remains is how often ecological conditions promote the spontaneous emergence of tight mutualistic symbiosis compared with alternative scenarios, such as enslavement by a host with or without a prior history of coevolution. ■

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Possible origins of mutualistic symbiosis. Scenarios for the origin of mutualistic symbiosis between a single host (the large individual) and multiple symbionts (small individuals), with (A, B, and C) or without (D) a prior history of coevolution. (A) Parasitism, followed by coevolutionary changes. Compensatory evolution in the host may lead to “addiction” of the host to the parasite (7). (B) Enslavement, followed by coevolutionary changes. (C) Commensalism, followed by coevolutionary changes. (D) Ecological fitting. A change in the ecological conditions is detrimental for two different species. However, the two species have complementary characteristics, immediately leading to a mutualistic symbiosis, as Hom and Murray experimentally demonstrate. We hypothesize that subsequent coevolution may then lead to tighter interactions, such as in extant lichens.

Emergent complex states in bilayer graphene

Experiments probe a wealth of exotic electronic behavior

By **Brian J. LeRoy** and **Matthew Yankowitz**

Symmetries play a critical role in physical systems. The famous Noether's theorem states that a continuous symmetry of a system has a corresponding conserved physical quantity. For example, a physical system that is invariant in position and time must obey conservation of linear momentum and energy, respectively. The breaking of symmetries has equally important consequences—some examples being the Higgs boson, superconductivity, and ferromagnetism. Electrons in graphene, a monolayer sheet of hexagonally bonded carbon atoms, exhibit an approximate fourfold symmetry due to the two equivalent atoms per unit cell and spin degeneracy. The unusual electronic transport properties that result when a magnetic field is applied reflect these symmetries (1–3). The two layers of carbon atoms in bilayer graphene provide an extra degree of freedom, making it an even richer system for complex electronic states to emerge. Using different measurement techniques, a trio of studies in this issue—by Kou *et al.* (4) on page 55, Lee *et al.* (5) on page 58, and Maher *et al.* (6) on page 61—have elucidated the nature of these exotic broken-symmetry states.

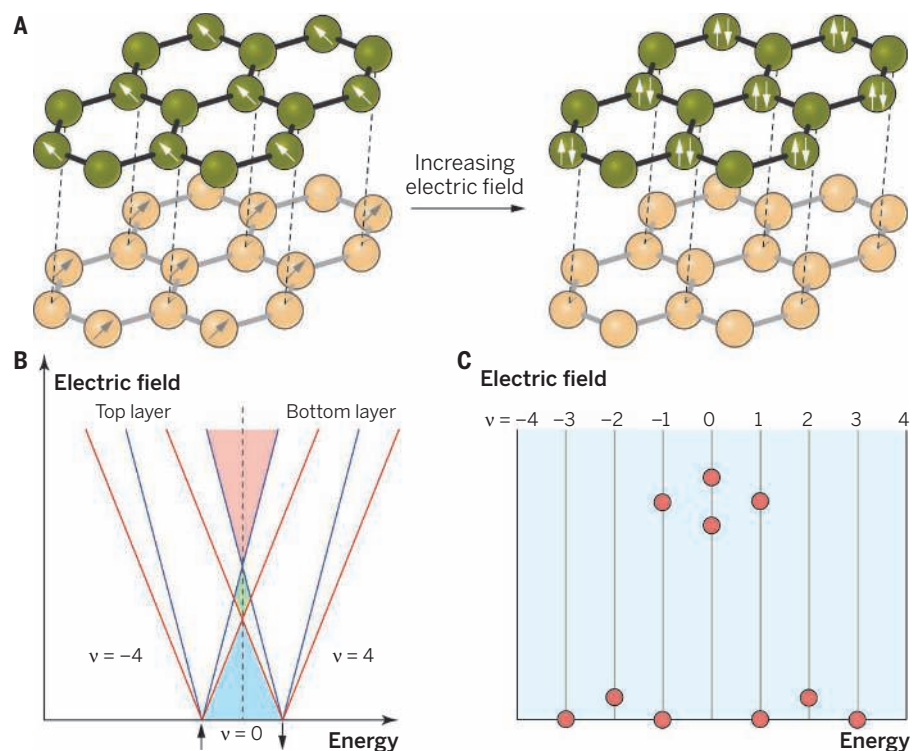
At small magnetic fields, bilayer graphene exhibits plateaus in Hall conductivity at values $\pm 4N(e^2/h)$ for $N \geq 1$, where N is the (integer) Landau level index, e is the electric charge, and h is Planck's constant. The factor of 4 arises from the spin and layer (also known as valley) degeneracy of bilayer graphene, and the missing plateau at $N = 0$ arises from an extra twofold orbital degeneracy due to the merging of the first two Landau levels at zero energy (7). Thus, plateaus occur at Landau level filling factors of $\nu = \pm 4, 8, 12$, etc., with a special eightfold symmetry around zero. The filling factor ν represents the ratio between the number of electrons and magnetic flux quanta. As the magnetic field is increased, the spin and layer degeneracies are broken, causing plateaus to appear at all integer filling factors. In very clean samples and at large magnetic fields, strong Coulomb interactions produce

additional quantized Hall states at fractional filling factors. Upon formation of these fractional quantum Hall states (FQHSs), the system is composed of fractionally charged quasiparticles that experience zero magnetic field (8). These quasiparticles can inherit the symmetries of their parent electrons, requiring even stronger symmetry-breaking terms before exhibiting broken-symmetry FQHSs. The competition between the strengths of these symmetry-breaking interactions helps determine the specific nature of the electronic ground state. These effects have been fairly well studied in monolayer graphene, but have thus far been largely unexplored in bilayer graphene owing to challenges in sample fabrication. Using various new device structures based on ultraclean bilayer

graphene on hexagonal boron nitride (9), these three groups have teased out these previously hidden states.

By performing scanning single-electron transistor measurements on an extremely clean bilayer graphene sample, Kou *et al.* observed a rich sequence of symmetry-broken quantum Hall states (QHSs) and FQHSs. Surprisingly, the sequence of FQHSs is not symmetric about filling factor $\nu = 0$, as is observed in monolayer graphene, but rather exhibits a $\nu \rightarrow \nu + 2$ pattern that is attributed to the extra twofold orbital degeneracy specific to bilayer graphene. Electron-electron interaction strength is expected to vary depending on the orbital polarization, weakening the strength of some of the typically observed FQHSs and resulting in an unusual sequence of states that is not electron-hole symmetric.

Lee *et al.* and Maher *et al.* add an additional ingredient to the mix: the ability to tune phase transitions in the QHSs and FQHSs with an external transverse electric field, which provides an independent symmetry-breaking term for the layer and orbital degeneracies. Lee *et al.* find particularly surprising behavior at $\nu = 0$, where four of the eight degenerate states in the



Bilayer graphene phase diagram. (A) Schematic of the bilayer graphene lattice indicating spin and layer configurations for the $\nu = 0$ state. At a small electric field, the system is in a canted antiferromagnetic phase (left); at a high field, it is in a layer-polarized phase (right); and at intermediate fields, it is in a coherent superposition of the two. (B) Schematic of the Landau level (LL) energies of bilayer graphene in an electric field. The LLs are initially spin-split because of a magnetic field, and become layer-polarized under an electric field. The orbital degree of freedom, indicated by color (red or blue), also evolves with electric field. For the $\nu = 0$ state, the canted antiferromagnetic (blue), spin-layer coherent (green), and layer-polarized (red) phases are color coded. (C) Experimentally observed phase transitions in the lowest LLs of bilayer graphene as a function of electric field. In general, phase transitions occur at crossings or splittings of LL lines shown in (B).

lowest Landau level are filled. At small electric fields, the ground state is spin polarized (canted antiferromagnetic), and above a critical field the ground state transitions to become layer polarized (see the figure, panel A). What is new is that the lifted orbital degeneracy due to the electric field induces a spin-layer coherent phase (coherent superpositions of Landau levels with the same orbital index but different spin and valley indices) separating the fully spin and fully layer polarized phases (see the figure, panels B and C). Maher *et al.* additionally track the electric field-induced phase transitions of FQHSs, observing weak electron-hole asymmetry reminiscent of the Kou *et al.* findings. The phase transitions in the FQHSs tend to match those observed in the parent QHSs, indicating that the fractionally charged quasiparticles inherit the same symmetries as their parent electrons and couple to symmetry-breaking terms with similar strength. The $\nu = 4/3$ and $5/3$ states are exceptions, exhibiting a phase transition at zero electric field where their parent $\nu = 2$ state does not, suggesting that the competition between ground state phases in the QHSs and FQHSs may be exceedingly complex.

It is remarkable that these three studies, using independent measurement and sample fabrication techniques, draw consistent conclusions about the nature of a system with so many degrees of freedom. Another recent study (10) examined FQHSs in high-quality suspended bilayer graphene and found rather different results, observing only two fully developed states at $\nu = -1/2$ and $-4/3$. The $\nu = -1/2$ state is especially intriguing, as it is expected to be non-Abelian (behaving as neither a boson nor fermion) and is not of the same origin as any of the FQHSs observed on the substrate-supported devices of Kou *et al.* or Maher *et al.* This result further demonstrates the wide variability of FQHSs in bilayer graphene, in contrast to monolayer graphene, in which the FQHS sequence is similar across all experiments. Though considerable work is necessary to fully understand the rich phase diagram of bilayer graphene, these studies open exciting pathways toward realizing and controlling the exotic emergent states. ■

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NEUROSCIENCE

Following the same nerve track toward different cell fates

Schwann cell precursors can become either neurons or glia

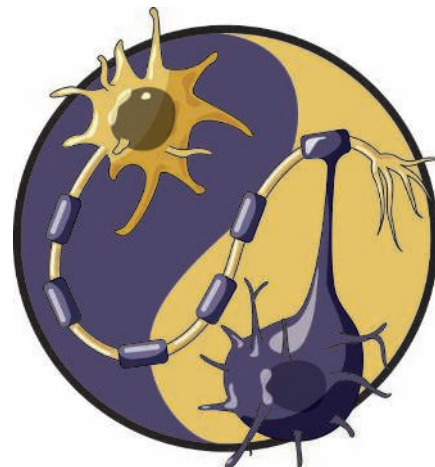
By Chaya Kalcheim¹ and Hermann Rohrer²

The autonomic nervous system controls the activity of internal organs such as the heart, lung, and gut, maintaining homeostasis of body functions in response to changing external conditions (1). It is composed of two antagonistic branches, sympathetic and parasympathetic. The former is essential for adapting to activity (i.e., fight-and-flight), whereas the latter is important at rest. Sympathetic neurons form ganglia along the body axis; parasympathetic ganglia are distributed all over the body. On page 87 and page 82 in this issue, Espinosa-Medina *et al.* (2) and Dyachuk *et al.* (3) challenge current views on how the parasympathetic nervous system is formed. These ganglia arise from progenitor cells that migrate along nerve fibers to peripheral targets. Known as Schwann cell precursors, these cells had previously been thought to give rise only to non-neuronal cells. Moreover, the nerve tracks include the very nerve fibers that ultimately innervate the parasympathetic neurons once they reach their destination and mature.

Both sympathetic and parasympathetic neurons derive from neural crest cells that emigrate from the neural tube (precursor to the brain and spinal cord) during early vertebrate development (4, 5). This migration to sites of ganglion formation and the molecular control of neuron differentiation have been well delineated for sympathetic ganglia, but the formation of parasympathetic ganglia has remained unclear (6, 7). Espinosa-Medina *et al.* and Dyachuk *et al.* demonstrate that parasympathetic ganglia are formed not by the aggregation of migrating neural crest cells but rather from Schwann cell precursors derived from neural crest cells. The Schwann cell precursors track along outgrowing axons from neurons in the developing hindbrain and transiently display a dual identity—part Schwann cell precursor and part parasympathetic neuron progenitor.

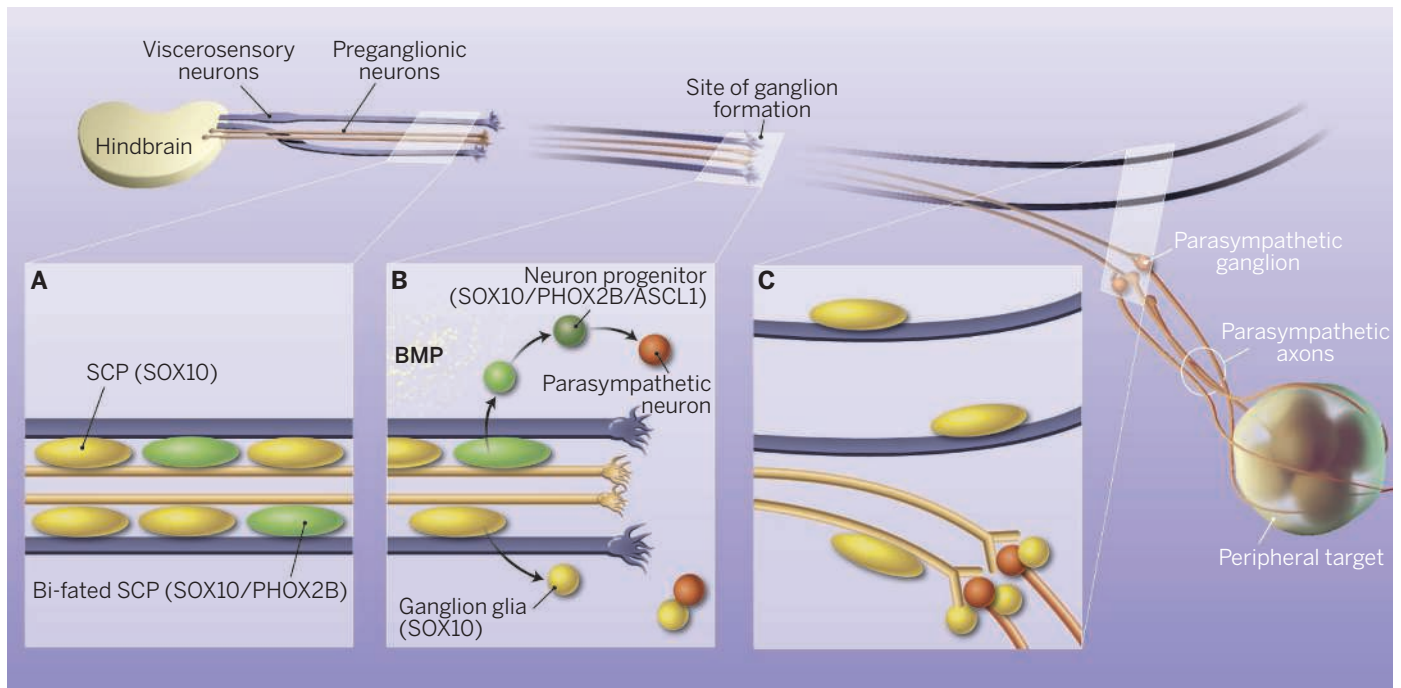
During mouse embryonic development, two parasympathetic ganglia (sphenopalatine and lingual) in the head are absent when a facial nerve that emanates from the hindbrain is partially eliminated (8).

This pointed to a role for cranial nerves in parasympathetic neuron development. Extending these findings to additional parasympathetic ganglia in the mouse head and trunk, Espinosa-Medina *et al.* used genetic methods to delete other cranial nerves—the glossopharyngeal nerve and/or the vagus nerve. The authors observed that the generation of parasympathetic neurons that constitute the otic ganglion—which stimulates a salivary gland—depends on the presence of the glossopharyngeal cranial nerve.



Formation of the cardiac ganglion—which innervates the heart—depends on the presence of the vagus nerve. Detailed analysis revealed that both parasympathetic ganglia arise from cells that accompany the cranial nerve fibers as they grow toward the site of parasympathetic ganglion formation. These migrating cells express the transcription factor SOX10, which indicates their crest cell origin and is a marker for future Schwann cells. During their migration, however, they also turn on the expression of another transcription factor, PHOX2B, which is a marker for autonomic neurons. They therefore assume a bi-fated precursor

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Bi-fated progenitors. (A) So-called Schwann cell precursors (SCPs) associate with outgrowing mixed viscerosensory and visceromotor nerve fibers from the embryonic mouse hindbrain. (B) At sites of future parasympathetic ganglion formation, these precursors detach from the nerve and change expression of certain factors to follow a neuron cell fate [likely in response to bone morphogenetic protein (BMP)] or a non-neuronal (Schwann cell or ganglion glia) fate. (C) Differentiated parasympathetic neurons innervate targets and are themselves innervated by visceromotor neurons from the hindbrain.

neuronal identity for neuronal and non-neuronal cells. At later stages, when the migrating cells that will become parasympathetic neurons begin to cluster and detach from the cranial nerve, SOX10 expression decreases (see the figure). Interestingly, the bi-fated precursor cells (expressing both transcription factors) are not restricted to cranial nerves but are also found in developing limb nerves where they generate a small ganglion composed of autonomic neurons that express PHOX2B.

Dyachuk *et al.* show that parasympathetic ganglia coalesce 1 or 2 days after the cessation of neural crest cell migration from the neural tube. This temporal gap suggested that these ganglia might not directly derive from neural crest precursors. Lineage tracing revealed that parasympathetic neurons in the mouse head are generated from cells associated with extending cranial nerves that express SOX10 and the transcription factor ASCL1. The latter is a marker for neuronal commitment. The authors also detected the neuronal marker PHOX2B in these cells and the dependence of parasympathetic ganglion formation on cranial nerves. These bi-fated precursor cells also expressed ERBB3, a receptor for neuregulin that is expressed by Schwann cells. In the ERBB3-deficient mice, both precursor cells and parasympathetic neurons are absent, indicating a transition from a bi-fated precursor to a parasympathetic neuron pheno-

type. Conversely, in the absence of ASCL1, the precursor cells remain as Schwann cells.

The findings of Espinosa-Medina *et al.* and Dyachuk *et al.* show that bi-fated precursors, known as Schwann cell precursors, that are present in viscerosensory cranial and vagus nerves are the source of neurons and non-neuronal cells. This extends previous findings showing that these precursors also give rise to skin melanocytes (9) that are restricted to the abdomen and limbs (10) and to endoneurial fibroblasts in the protective connective tissue that surrounds nerves (11). Thus, early developing nerves represent a convenient source of, and pathway to deliver, progenitor cells to distant sites. Interestingly, parasympathetic ganglion development relies completely on this principle, but the presence of bi-fated progenitors (expressing PHOX2B and SOX10) is not restricted to nerves containing preganglionic parasympathetic nerve fibers (2). The discovery of an autonomic ganglion (expressing PHOX2B) along a limb nerve in the developing mouse suggests that parasympathetic ganglia may form transiently and may be eliminated in the absence of preganglionic innervation (12).

Espinosa-Medina *et al.* and Dyachuk *et al.* propose an elegant solution for the wiring of preganglionic autonomic neurons to their peripheral postsynaptic parasympathetic targets. It is yet unclear what signals con-

trol the fate switch in the migrating bi-fated precursor cells, how PHOX2B expression is regulated in these cells, what factors elicit detachment from the cranial nerve and subsequent neuron differentiation, and how progenitors become restricted to a parasympathetic neuronal fate rather than a sympathetic neuronal fate. It would also be interesting to clarify whether individual Schwann cell precursors display stem cell-like properties to generate parasympathetic neurons, melanocytes, and endoneurial fibroblasts or whether they are a heterogeneous cell population that can produce specific combinations of cell fates depending on the spatiotemporal context. ■

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CLIMATE POLICY

IPCC lessons from Berlin

Did the “Summary for Policymakers” become a summary by policy-makers?

In April in Berlin, governments approved the third of three reports comprising the fifth assessment report (AR5) of the Intergovernmental Panel on Climate Change (IPCC). The report from Working Group 1 (WGI) made clear that human impact on climate change is almost certain. WGII showed that impacts of climate change are evident and poised to worsen. WGIII focused on how to mitigate the emissions that cause global warming (1).

Although the underlying technical report from WGIII was accepted by the IPCC, final, heated negotiations among scientific authors and diplomats led to substantial deletion of figures and text from the influential “Summary for Policymakers” (SPM). The deleted content focused largely on historic emissions trends analyzed by

POLICY country income groups and international cooperation. IPCC authors are instructed to be policy-relevant, without being policy-prescriptive, and the SPM is intended to balance governmental and scientific input. But some fear that this redaction of content marks an overstepping of political interests, raising questions about division of labor between scientists and policy-makers and the need for new strategies in assessing complex science. Others argue that SPM should explicitly be coproduced with governments.

To promote discussion of whether and how to reform IPCC in advance of the 15th Meeting of the Conference of the Parties to the UN Framework Convention on Climate Change (UNFCCC) in November 2015, *Science* invited several WGIII members to share their perspectives on what happened in Berlin and what it means for the IPCC and climate policy.

By **Brad Wible**



Getting serious about categorizing countries

By **David G. Victor**,^{1,9} **Reyer Gerlagh**,^{2,9} **Giovanni Baiocchi**,^{3,10}

A central finding of WGIII is that growth of income has been the largest single driver of emissions. Governments accepted that finding at the global level, where it is safe to discuss generalities because no country is in the spotlight. But WGIII also showed how different categories of countries contribute to global emissions (charts 1 to 3). We explain what was lost when these figures were cut from the SPM.

Since the industrial revolution, today's highly industrialized countries have been the main contributors to greenhouse gas (GHG) emissions (chart 1). But over the past decade, their emissions have been roughly flat, whereas emissions from upper-middle-income countries (UMCs) have risen rapidly (chart 2A). The central implication is that international climate policy needs to update how it categorizes countries. In the early 1990s when the UNFCCC was created, countries were divided into two categories—industrialized nations (Annex I), and the rest (non-Annex I). As non-Annex I countries' emissions have soared, their participation in future climate agreements is essential.

Getting serious about categorizing countries will reopen old, but unavoidable, political fissures. Industrialized countries—which, on average, still have the highest per capita emissions—must do more to

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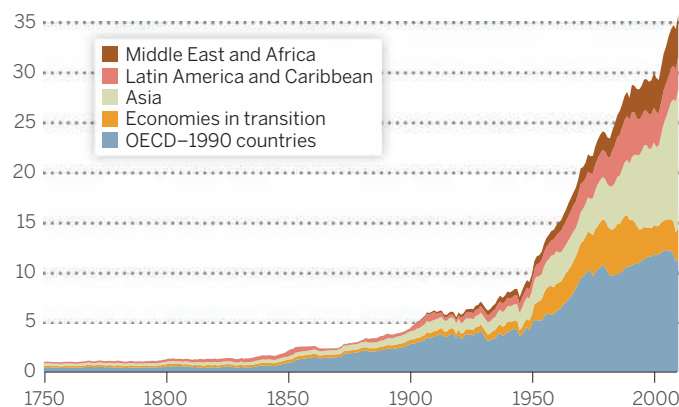


Energy consumption drives richer countries' emissions, particularly coal, as in China.

cut emissions. Emerging economies must accept that they account for most of the recent and expected emissions growth. Unless both groups take concerted action, stabilizing the climate will be impossible.

There are many schemes for grouping countries. The best choice depends on the purpose of the analysis. The full WGIII report uses several schemes, but in the SPM a central purpose was to minimize variation in emissions within each category, and thus, it grouped countries according to underlying economic drivers of emissions. The writing team selected the latest ver-

Total anthropogenic CO₂ emissions (Gt/year)



Historical view of CO₂ emissions from fossil fuel combustion, flaring, cement, forestry, and other land use. Areas represent cumulative emissions. OECD, Organization for Economic Cooperation and Development. [Reproduced from figures TS.2a and 5.3 (1)]

sion of a World Bank scheme widely used by experts in economic growth and better suited to explaining variations in emissions than the Annex I scheme. Nonetheless, there remains huge variation within these categories (chart 2B), particularly for the least-developed countries, where the greatest emissions are related to agriculture and land use. In richer countries it is energy consumption that drives most emissions. That key finding was also cut from the SPM. And any scheme is sensitive to the classification of China's big economy, which was upgraded to UMC status in 2010. Although income is an important driver (chart 2C), within income categories, many other factors, including policy, lead to highly variable emissions. Some UMCs have per capita emissions about equal to the most frugal industrialized countries whose income levels are a multiple.

These figures also reveal the changing structure of the economy, measured in energy intensity (emissions per dollars of output, chart 2D). As economies mature, they become more efficient. But this improvement in energy intensity has not been fast enough to offset the overall effect of growing economies. That's why policies are needed and help explain the wide variation in emission intensity. Some countries have adopted aggressive policies, whereas others have done almost nothing.

High variability in the factors that determine emissions has implications for the design of climate agreements. If a wider array of countries is to be engaged in the global effort to control emissions, a single schedule of binding targets and timetables

is unlikely to work. International agreements must be flexible to accommodate different national circumstances, including uncertainty in economic development—a subject of much scientific research also cut from the SPM.

Studies on effects of trade and globalization have tracked emissions “embodied” in products that are traded across borders. For the first time, IPCC presented adjusted emission statistics showing how territorial and consumption-based accounting systems lead to very different pictures. But because governments couldn't agree on how to group countries, all WGIII findings about embodied emissions were cut from the SPM. What was lost is shown in chart 3.

Embodied carbon has increased dramatically since 1990. Current international policy approaches, which ignore trade, create strong incentives for countries to sit outside climate agreements and to take a free ride on benefits, not only in terms of climate change mitigation but also because of industry relocation. Better accounting would allow better incentives, such as border taxes and other adjustments for trade. Sophisticated rules within the World Trade Organization can keep countries from abusing such measures for protectionist purposes. Some countries are already experimenting with such approaches—the European Union (EU), for example, is extending its emission rules to cover foreign aircraft that use EU airports.

Most talk of IPCC reform is focused on how it must change. For example, the IPCC should shift from infrequent big reports to more timely shorter and focused studies; the review process could be streamlined. But IPCC is a government-controlled process. Its line-by-line approval of the SPM yields the lowest common denominator of what is scientifically accurate and not too toxic for governments. A small number of countries can block findings that a large number of scientists working over many years with extensive review have agreed are robust.

Disentangling IPCC from politics is impossible, especially where IPCC engages social science research that has policy-relevant conclusions. Yet IPCC as a scientific body can sharpen its messages by focusing more attention on the author-approved technical and policy summary documents.

The most important rethinking is needed outside the IPCC process. When the IPCC began in the late 1980s, it eclipsed many different national climate science assessments. We predict and encourage a return to that multiplicity of assessments. This allows greater tailoring of assessments to local circumstances, values, and priorities. It will also encourage fuller diversity of scientific views. The IPCC will continue to have an important role to help stitch together many diverse assessments into a global perspective, while focusing on questions of special diplomatic importance such as impacts on the least-developed countries. ■

10.1126/science.1255302

Political implications of data presentation

By Navroz K. Dubash,^{4,10} Marc Fleurbaey,^{5,9} Sivan Kartha^{6,9}

What is the appropriate balance between scientific analysis and governmental input in the IPCC? Claiming government overreach and calling for greater insulation of the process come from a misleadingly simple interpretation. Such insulation would likely diminish the policy relevance of the SPM. The SPM is “approved” by governments, not merely “accepted” as is the main report, which invests it with an important measure of governmental ownership. An approval process is worth preserving, as it is precisely what makes the IPCC distinct from any number of technical reports. We explore an alternative vision for articulating science and politics at the IPCC.

As the parties work toward a next phase in the global climate treaty to be agreed on in late 2015, perhaps the single most contentious issue is that of country groupings, as they are directly linked to national commitments under the UNFCCC. IPCC findings perceived to influence revision of groupings were thus liable to trigger a confrontation in Berlin.

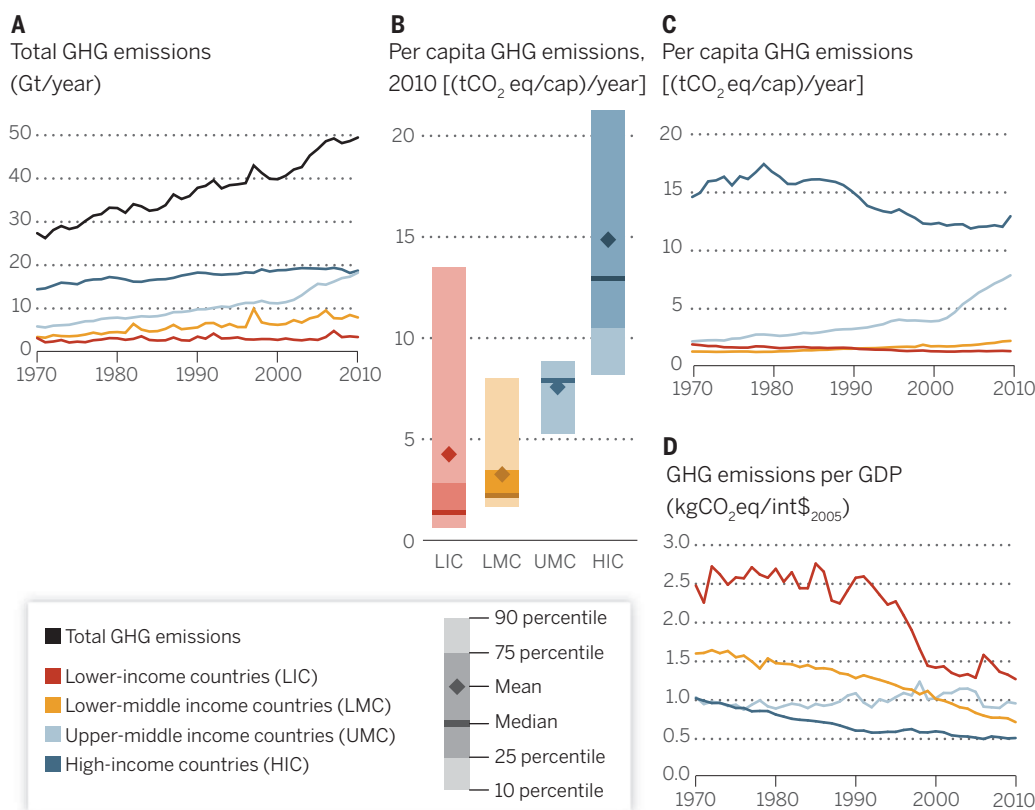
As income growth has been identified as a key driver of emissions growth, income groups appear a natural analytical tool, although the full WGIII report most frequently uses traditional regional groups, as well as sectoral disaggregation. At least two challenges confront any grouping approach in the context of an SPM.

First, in the negotiation con-

text, analytical groupings are inevitably interpreted as to implications for political groupings. Imposing political perceptions in the context of a scientific assessment might be seen as intrusive. However, in producing an SPM with explicit government buy-in, engagement on such issues is inevitable. The question is how to make it productive.

Second, grouping heterogeneous and rapidly changing countries into a few categories necessarily elides relevant information. Country groups contain large variances (chart 2B), aggregate statistics can be dominated by a few countries, and many alternative groupings are possible. Small changes in number of groups, thresholds between groups, types of characteristics, and reference period can lead to large changes in findings with substantial political implications.

For example, chart 2 categorizes countries according to their current (2013) income, showing that UMCs accounted for three-quarters of global emissions rise from 2000 to 2010. A political interpretation of this may be that the UNFCCC must update its country groupings to reflect the role of UMCs and to impose commensurate emission limits. However, the same representation based on countries' income in a year during the 2000–2010 decade (e.g., 2005) would show that three-quarters of the 2000–2010 global emissions rise arose in lower-middle-income countries, rather than UMCs, and a further 20% in low-income countries. A corresponding political interpretation might stress the need for financial and technological support to enable countries to develop along a lower-carbon pathway through low- and lower-middle income stages. The two representations would be equally faithful to underlying data, but also equally synthetic and



Anthropogenic GHG emissions, by country income group: Over recent decades, emissions from different groups have displayed markedly different patterns. Groups based on World Bank 2013 country income classification. (A) Annual total emissions. [Reproduced from figures TS.4 and 1.4 (1)] (B) Distribution of annual per capita emissions. [Reproduced from figures TS.4, 1.8c, and 5.19 (1)]. (C) Annual median per capita emissions. [Modified from figures TS.4 and 1.4 (1)] (D) Annual median emissions per unit of gross domestic product. GDP expressed in 2005 international dollars. [Computed from economic data in figure 5.15 using country income groupings and emissions reported in chart 2 (1)]



Land use, like deforestation in Indonesia, drives emissions in lower-income countries.

incomplete, and differ markedly in the political extrapolations to which they ostensibly lend credence.

What happened with two other sections of the SPM suggests that the outcome of political debate on scientific findings is contingent. The framing section of the SPM deals with very contentious issues about the need for cooperation in mitigation and technology, as well as the centrality of equity in burden-sharing and in the evaluation of costs and benefits, among others. Yet, agreement was reached on this section—indeed, it was expanded rather than truncated. This was due, in part, to serendipity; the framing section was taken up for discussion early in the approval process, which allowed 4 days for discussion and iterations of text, and that enabled convergence on what authors sought to convey and the political-legal sensibilities brought by country delegates.

In contrast, discussion of another section, on international cooperation, produced much shorter text and simplified content, stripped of controversial elements. This was due both to the short time available for this discussion and to a general spirit of contention after the removal of several figures.

What does this suggest about the IPCC process? The main IPCC report should be focused on establishing and presenting scientific facts. But seemingly technical choices can crystallize into value-laden political conclusions, particularly given tight word and time limits. It is more productive for authors to be aware of alternative political interpretations of their concepts and findings and to factor these into representations of data, than to strive, unrealistically, to ignore political concerns. This requires rethinking the SPM as a coproduction process in which salient political discussions are connected to relevant scientific material. Changes in the SPM writing process can help: creating more channels and space for dialogue before the pressure cooker of a time-limited approval session, ensuring strict continuity and transparency between drafts, and treating author diversity of perspectives as an asset.

The process of coproducing a portion of the IPCC with governments is what lends the IPCC its credibility as a voice that is of scientists, but with more weight for policy. Calls for insulation from politics risk undermining the relevance of the SPM. ■

10.1126/science.1255734

Mapmakers and navigators, facts and values

By Ottmar Edenhofer^{7,8,11} and Jan Minx^{7,12}

Discussion around the SPM approval process has been ill-framed, raising questions of scientific credibility. Although changes were made during the government approval process, author involvement preserved consistency with the science in the underlying report. Even if scientists desired more far-reaching consensus in some areas, the government-approved SPM remains scientifically credible. The underlying report and Technical Summary were unaffected by changes made during SPM approval. The report provides a “living map,” drawn in a social learning process between scientists (mapmakers) and policy-makers (navigators), to be used to traverse the largely unknown territory of climate policy.

The SPM successfully provides a comprehensive, policy-relevant assessment of mitigation pathways to alternative climate goals in terms of underlying technological, economic, and institutional requirements. This was the key expectation for the WGIII report. But the approval process also revealed limitations of the IPCC in processing scientific knowledge with immediate relevance for negotiations, in particular, ex post assessment of progress made to date in climate change mitigation efforts.

Government concerns over the grouping of countries arose despite major efforts to provide a balanced assessment: The SPM section on emission trends and drivers that entered the approval plenary offered more analytical perspectives than any prior WGIII assessment. As is common scientific practice, countries were grouped in different ways depending on the question assessed. The SPM focused on why emissions have been growing as countries develop and how these patterns have shifted over time. The income-based classification was selected for its scientific merits to provide statistically robust insights and was subsequently used to analyze historic emission trajectories from multiple perspectives.

The fear of some governments, presumably, was that approval of any country classification other than that currently used under the UNFCCC could be disadvantageous in upcoming negotiations for a

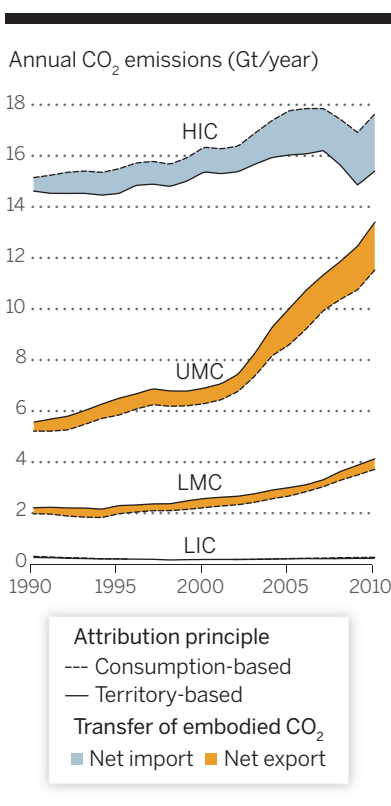
new international climate regime. But traditional UNFCCC classifications (Annex I versus non-Annex I) are not useful for scientific questions of the type considered in the SPM, because they show too much variation for insightful analysis. In the end, neither income-based nor any other alternative classification used in the underlying report was acceptable to all governments. Thus, figures and associated text had to be removed from the SPM.

Discussions on historic emission trends and drivers were part of a broader conflict over material that could be related to past performance of countries or regions in mitigating climate change. Such ex post analyses of international climate policies received new importance in the AR5, as the first commitment period of the Kyoto Protocol came to a close during the report cycle. Yet what remains in the SPM on the topic of international cooperation is limited to factual statements describing the status quo without any critical ex post evaluation of these policies. In our view, a reasonable summary of ex post policy analysis that considers different evaluation criteria is advantageous for negotiations.

Scientific concepts required for ex post policy evaluation as such do not have legal implications, nor should they predetermine future negotiations. Legal consequences require explicit reference to ethical norms like responsibility, capacity, or burden-sharing. During the Berlin approval session, government parties to the IPCC failed to agree on a reasonable way to analytically distinguish scientific analysis from potential political and legal interpretations. The SPM thus lost a valuable evaluation of policy performance that could have allowed for an informed, reasonable debate on mitigation. One notable exception was the characterization of climate change as a global commons problem.

The question of how the IPCC can provide ex post evaluation of climate policies in a SPM, which is approved line by line, will not dissolve after the AR5. As new mitigation policies are implemented over time, ex post evaluation of policies will become an increasingly important component of future IPCC reports.

The main challenge for the future of the IPCC is not one of orga-



Attributing CO₂ emissions from fossil fuel combustion based on sites of production as compared with sites of consumption. Emissions “embodied” in traded products have increased dramatically since 1990, with high-income countries net importing, all others net exporting. Groups based on World Bank 2013 country income classification. [Modified from figures TS.5 and 1.5 (1)].

nization and procedures. The real challenge is how the IPCC conducts assessments and deals with entanglement of facts and values at the science-policy interface. Nevertheless, factual and normative statements can usually be analytically distinguished. The SPM sections on framing and ethics and the underlying framing chapters, novel in the IPCC, offer a first blueprint for rationally discussing value judgments that often accompany scientific concepts and different perspectives. Presenting alternative pathways in ex ante analyses and multiple perspectives in ex post analyses is key if the IPCC wants to present meaningful assessments of human response options to climate change in the future.

Acknowledgment that a rational debate of facts and values is possible lies at the heart of any relevant assessment. This enlightened approach came under attack in Berlin. The IPCC has a choice: Either run the risk of becoming less and less policy-relevant or find a way for

ex post assessment of climate policies to be clearly presented in government-approved summary documents. This should be done on the basis of clear understanding of legitimate roles of scientists as mapmakers and policy-makers as navigators. From such common understanding, the IPCC can further inform international climate policy without prescribing and predetermining future negotiations. How to further develop the art of assessment making should be at the heart of the ongoing discussions on the future of the IPCC. ■

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10.1126/science.1255998



Shaking up volcanoes

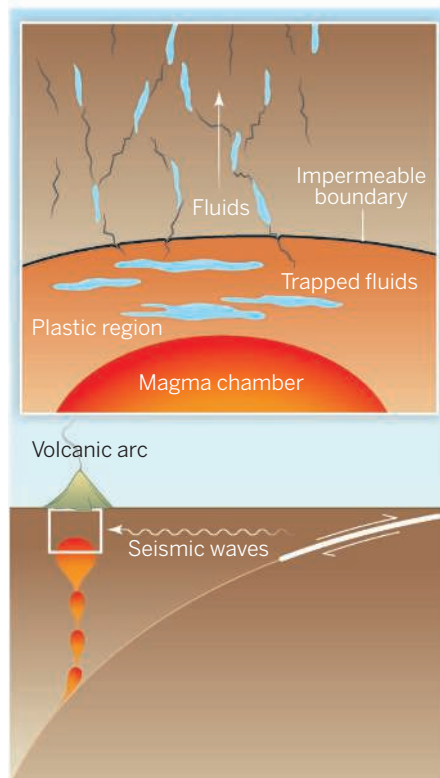
Data from a Japanese seismic network elucidate how large earthquakes may disrupt volcanic systems

By **Stephanie G. Prejean** and
Matthew M. Haney

Most volcanic eruptions that occur shortly after a large distant earthquake do so by random chance. A few compelling cases for earthquake-triggered eruptions exist, particularly within 200 km of the earthquake, but this phenomenon is rare in part because volcanoes must be poised to erupt in order to be triggered by an earthquake (1). Large earthquakes often perturb volcanoes in more subtle ways by triggering small earthquakes and changes in spring discharge and groundwater levels (1, 2). On page 80 of this issue, Brenguier *et al.* (3) provide fresh insight into the interaction of large earthquakes and volcanoes by documenting a temporary change in seismic velocity beneath volcanoes in Honshu, Japan, after the devastating Tohoku-Oki earthquake in 2011.

Large earthquakes can affect volcanoes in multiple ways. The most far-reaching effects result from radiated seismic waves. Unlike the immediate and long-lasting static changes to Earth's stress field in the region surrounding an earthquake's rupture plane, seismic waves can disturb Earth's crust much farther away. Miyazawa (4) has shown that passing seismic waves from the Tohoku-Oki earthquake triggered small earthquakes throughout Japan, including at some volcanic centers on Honshu.

In the past two decades, a wealth of research has documented subtle changes in local seismicity, deformation, and hydrology at volcanoes after large earthquakes (1, 2). Models developed to explain the interaction involve stress changes across local fractures and magma storage regions, activation of hydrous or magmatic fluids, and/or localized deformation. For example, in the hydraulic surge (see the figure) (5) and fracture unlogging (6) models, shaking from seismic waves breaches impermeable boundaries that separate compartments in the crust beneath volcanoes. This process allows high-pressure fluids to migrate along pressure gradients up and away from source regions. Because migrating fluids can in-



Seismic waves may trigger fluid flow at volcanoes.

In the hydraulic surge model (7), high-pressure pore-fluids are trapped in impermeable plastic regions of the crust. Seismic waves rupture impermeable boundaries, allowing fluid migration. Brenguier *et al.* document a temporary increase in pore-fluid-pressure beneath volcanoes in Japan following the great Tohoku-Oki earthquake.

duce earthquakes, some have speculated that hydraulic surges cause dynamically triggered earthquakes at volcanoes. These and other models cannot be verified, however, without better hydrologic and geodetic monitoring to track pore-fluid pressure and other crustal properties. Brenguier *et al.* provide a key to this fundamental problem using a common and widespread data source known as seismic noise.

Only in the past decade has seismology fully embraced the idea of turning seismic noise into usable signal. Campillo and Paul's groundbreaking demonstration of obtaining signal from seismic noise (7) used only three station pairs, but soon afterward, Shapiro *et al.* (8) transformed the developing field by using hundreds of station pairs to map the distribution of seismic velocity over all

of southern California. The method, now known as ambient noise tomography, has provided unprecedented insights into the structure of Earth's crust.

In a similar vein, Brenguier *et al.* build on previous studies of time-dependent velocity changes at individual volcanoes (9) but expand the scope to the entire volcanic arc of Honshu, Japan. By processing thousands of station pairs from one of the highest-quality seismic networks in the world, the Japanese Hi-net array, they create a map of a new measurable quantity: seismic velocity susceptibility, or the ratio of subsurface velocity change to applied dynamic stress. Velocity susceptibility is an optimal measure of earthquake-induced fluctuations in crustal properties because changes such as crack opening and fluid intrusion can decrease seismic velocities.

The authors show that, in the days following the Tohoku-Oki earthquake, the most negative velocity susceptibilities followed a distinctive north-south trend along the volcanic central portion of Honshu. Why did the strongest effect not occur closer to the earthquake? Brenguier *et al.*'s explanation rests on the fact that volcanoes overlie localized regions of high pore-fluid pressure. These high pressures, the same ones that may help drive volcanoes into eruption, react more strongly to earthquake shaking than the surrounding crust and in turn control the spatial distribution of susceptibility.

Mapping seismic velocity susceptibility opens up the prospect of detecting changes in crustal properties, such as subsurface pore-pressure conditions, over wide swaths of Earth's crust. Further insight will come from large-scale measurement of time-dependent velocity changes with other spatially dense and extensive seismic arrays, such as USArray. In weighing models for earthquake-volcano interactions, understanding both changes in crustal properties and their time dependence is critical. Thus, studies like that of Brenguier *et al.* will help to reveal the mechanics of dynamic processes within volcanoes and allow us to more accurately address the vital question of how earthquakes affect volcanoes. ■

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BOOKS *et al.*

HISTORY OF SCIENCE

Mind, politics, and the academy

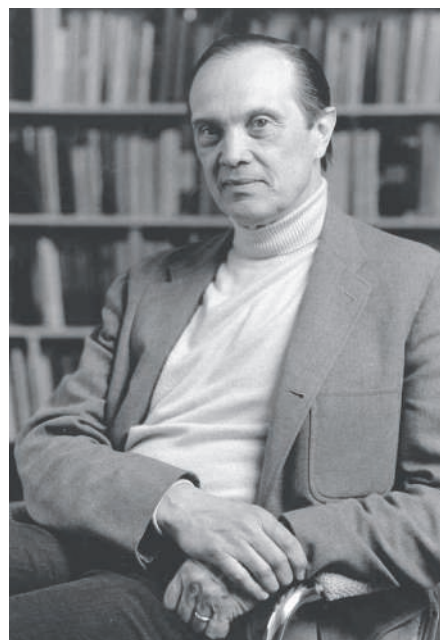
By Joel Isaac

In the years following the Second World War, social scientists in the United States devised a series of tests for identifying what they termed the “authoritarian personality.” What kinds of character traits made persons susceptible to fascist ideology? And what habits of mind correlated most closely with democratic rule? No one doubted the urgency of these questions. Much blood had been shed in the defeat of fascism in Europe. Democracy, meanwhile, had become the default regime of the West, yet its long-term viability was far from assured, given the ease with which the enfranchised masses had been won over by charismatic authoritarians in the 1930s. How American social scientists set about solving this puzzle is the subject of Jamie Cohen-Cole’s rich and illuminating study.

The Open Mind can be read as a history of psychology and in particular of the construction of the research program of cognitive science—a field now associated with the names of George Miller, Noam Chomsky, and Jerome Bruner. But the book can equally be read as a study of the political culture of mass democracy in the United States or as a history of the American research university and the culture that sustained it. All of this is by design, for Cohen-Cole’s central thesis is that these three levels—of the human mind, the academic mind, and the American mind—were connected and mutually reinforcing.

To see why this conjunction of science, academia, and politics became, for a time, a potent solution to the problem of justifying mass democracy, we can turn to the classic 1950s studies of the authoritarian personality. The idea animating these investigations was that one could measure inclination toward authoritarian behavior and pick out those elements of personality that made for good citizens. One group of social psycholo-

gists even constructed a psychometric scale to record the propensity toward fascism. A variety of tools were deployed to measure an individual’s place on the “F-scale.” The most important was the questionnaire. Subjects’ responses were correlated with “High” or “Low” placements on the scale. In one test reported by Cohen-Cole, subjects were asked to “name people they admired.” The researchers identified the first group of responses (including “Whitman, Pushkin, Beethoven, Voltaire, Bertrand Russell, Comte, Maimonides, Confucius, Sir William Osler, Freud, [and] Pestalozzi”) with democratic character, because they indicated an esteem for “intellectual, aesthetic, and scientific achievement, social contribution, and democratic social change,” while the latter group (including “Marshall, MacArthur, Lindbergh, the Pope, Henry Ford, Washington, Teddy Roosevelt, Kate Smith, [and]



Cognitive scientist George A. Miller.

Bing Crosby”) signaled an authoritarian valuation of “power and control, conservative Americana, etc.” There was one obvious problem with this test, which critics were quick to point out: the first list of names was composed of those whose work would be known to, and admired by, intellectuals and the highly educated. As Cohen-Cole drolly notes, “the individuals admired by democratically minded Americans” were, in the words of one critic, “largely unknown to non-intellectuals.”

Somewhat astoundingly, however, this was an assumption the investigators would have been comfortable with. For the ideal type of democratic character was, in effect, read off from the intellectual virtues

The Open Mind Cold War Politics and the Sciences of Human Nature

Jamie Cohen-Cole

University of Chicago
Press, 2014. 405 pp.

increasingly associated with a certain kind of academic intellectual. These virtues included creativity, flexibility, openness to new ideas, autonomous thinking, tolerance of ambiguity or complexity, and an ability to work across cultural or institutional dividing lines. In short, the kind of open-mindedness associated with leading figures in the scientific disciplines, and especially in the social sciences, became a model for the democratic citizen more generally. Similarly, the narrow-minded, gate-keeping mentality that defined the views of those prone to authoritarianism in politics was projected onto the “dogmatic” defenders of a narrowly empirical form of psychology known as behaviorism. For the early proponents of cognitive science, behaviorists like B. F. Skinner and Stanley Smith Stevens were not just uncongenial colleagues: they were persons who could not recognize the creative, reflexive character of human cognition, which they sought to reduce merely to observable correlations between stimulus and response. The cognitive psychologists’ repudiation of behaviorism was therefore pervaded by normative notions of what made for the best scientific minds and for democratic personalities.

One of the most powerful features of Cohen-Cole’s account of the emergence of cognitive science is precisely that it refuses to reduce cognitive psychology to democratic politics. The arrows of social causation point in several directions: from a set of concerns about American society in an age of specialization and mass conformity to the academy, but also from cognitive science to the interdisciplinary culture of the postwar university and then outward toward the uncertain landscape of Cold War era politics. While no science exists in a bubble, political culture, in turn, is not impervious to changing scientific conceptions of human nature. *The Open Mind* also shows how the consensus on the value of the open mind helped to create a backlash from the New Left and New Right that eventually rent it asunder in the 1960s and 1970s. Anyone who wants to know about American democracy in the postwar era, and the special place of psychology within it, will profit enormously from reading Cohen-Cole’s excellent study. ■

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Of humans and mathematical symbols

By Gaia Donati

If the neighbor's children came to your door and asked you to help them with the practice of cosmic art, how would you react? Before you utter an improbable excuse out of embarrassment, you may be interested to know that, back in the 16th century, algebra was sometimes referred to as the art of determining the "thing" (*cosa* in Italian), i.e., the unknown root of a given equation—hence the expression cosmic art.

Joseph Mazur's *Enlightening Symbols: A Short History of Mathematical Notation and Its Hidden Powers* is a figurative cabinet rich with such curiosities. However, the book is more than a collection of fun facts about mathematics and the evolution of its language. A few pages into the introduction, Mazur remarks that "mathematics notation did not become really symbolic before the sixteenth century." This is a fairly strong statement. We—living in the 21st century—have inherited a set of symbolic tools crafted over the millennia of human history. *Enlightening Symbols* retraces the winding road that has led to the way we now teach, study, and conceive mathematics.

To guide readers through this mathematical journey, Mazur starts with some fundamental questions: When and where did numerical writing begin? How did the Western system of numerals—together with the problematic zero—evolve from Babylonian clay tablets and Chinese rod counting to present-day mathematics textbooks? What insights do symbols bring to mathematical thinking? And at what price, if any?

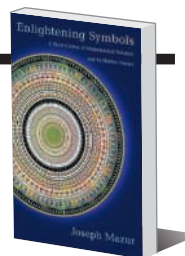
As Mazur sets out to address such easily overlooked aspects, he also reaffirms their relevance, showing how rich and complex the topic is. Mathematical writing appeared before any literary written composition, yet we lack a certain date for the first evidence of numerical writing. The further one delves into the texts and scholarly debates, the more one realizes that there is no smooth curve along which each modern mathematical symbol can be associated with an originator and a given point in time.

One could legitimately suspect that the missing information was stored in documents misplaced, destroyed, or badly preserved and hence illegible. While this is

Enlightening Symbols A Short History of Mathematical Notation and Its Hidden Powers

Joseph Mazur

Princeton University Press,
2014. 309 pp.



sadly true, it may come as a surprise to find out that uncertainty also pervades the study of available, well-known sources. It is in this context that Mazur draws the reader's attention to Diophantus's *Arithmetica* and Fibonacci's *Liber Abaci*. There is an ongoing dispute as to whether the Italian mathematician pioneered the introduction of Hindu-Arabic numerals in Europe. Mazur eventually observes that, leaving the debate aside, Fibonacci was undoubtedly "an excellent expositor of new and difficult concepts," addressing tradesmen of the early 13th century to advocate a novel numerical system. This is a fair conclusion, but a few additional excerpts from the *Liber Abaci* may have helped readers form their own opinions. On the other hand, the chapter dedicated to the *Arithmetica* illustrates how the translation and copying of ancient

texts are as crucial as successful preservation through the centuries. (The original *Arithmetica*, probably written between the 1st and 3rd centuries, does not survive.) Translation is a delicate art in itself; did Diophantus introduce a symbol for minus, or was that the creation of an overworked scribe?

After finishing the book's first two sections—one covering numerals, the other focused on algebra—readers will be left with the impression that a common denominator links every open question, doubtful attribution, and unknown origin. I would call this the human factor. The history of mathematical notation is a human journey of intuition, rigor, and abstraction—not to mention rediscovery. Referring to the evolution of number, mathematician Tobias Dantzig described it as a "profoundly human story" (1).

It is gripping to read about the contributions, big or small, of so many human minds—from real game-changers such as René Descartes, Gottfried Leibniz, and Isaac Newton to less-familiar names such as Robert Recorde (who introduced our equal sign) and François Viète (who used dedicated letters to designate knowns and unknowns in a polynomial equation, an idea later formalized by Descartes).

Following this exploration of human imagination, the book's third and final section presents a brave attempt to go beyond the symbols. Here, Mazur considers "whether mathematics is primarily visual-spatial, or predominantly linguistic." Although the arguments are not always linear or easy to follow, they draw on a number of interesting experimental results investigating mathematical and symbolic cognition.

Thanks to Mazur's playful approach to the subject, *Enlightening Symbols* offers an enjoyable read. Is it really true—as theoretical physicist Jim Gates claimed in a talk that I recently attended—that we are the only species on Earth that produces mathematics? Perhaps. What is surely less debatable is that the human journey through mathematical concepts (and correlated notations) is far from being over. ■

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Competing computers. This woodcut from Gregor Reisch's *Mararita Philosophica* (16th century) depicts Arithmetic presiding over a contest between Pythagoras at the counting board and Boethius calculating with Indian numerals.

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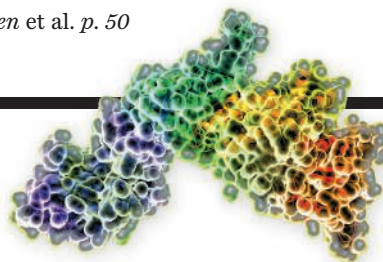
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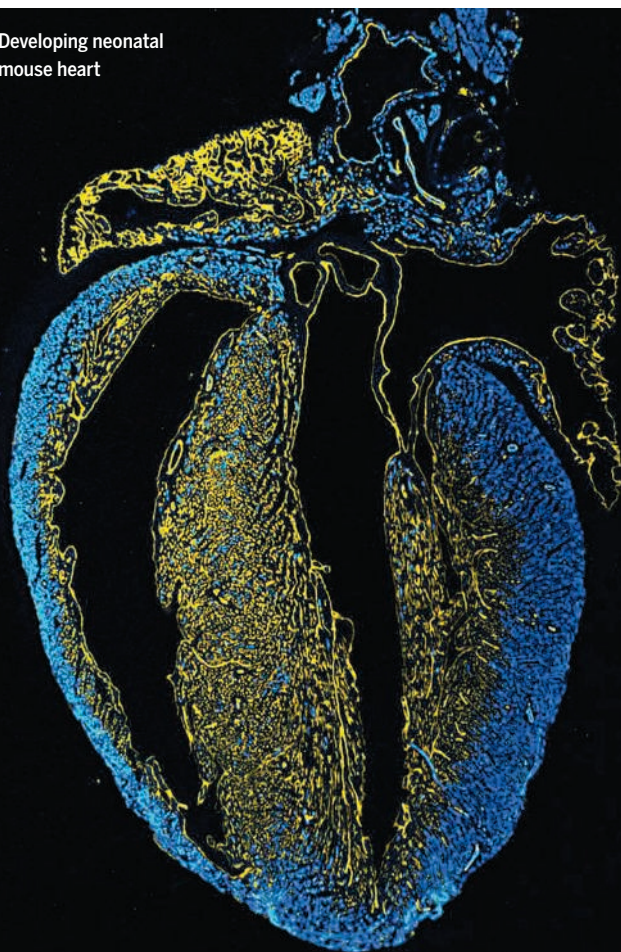
Edited by **Stella Hurtley**

A handful of individual enzyme molecules determine signaling output

Iversen et al. p. 50



Developing neonatal mouse heart



VESSEL FORMATION

The heart needs blood vessels, too

For the newborn heart to grow quickly, the heart's own blood vessels must grow as well. Researchers have assumed that preexisting fetal coronary vessels expand to cause this postnatal coronary vascular growth. Instead, Tian *et al.* now show that, for the most part, brand-new blood vessels form within the neonatal heart (see the Perspective by Burns and Burns). This ability to produce new coronary blood vessels after birth may one day help researchers work out how to promote cardiovascular regeneration after injury or disease. — BAP

Science, this issue p. 90; see also p. 28

TRANSPLANTATION

Toward safer bone marrow transplants

People who receive bone marrow transplants often develop graft-versus-host disease, in which immune cells from the transplanted bone marrow attack the patient's body. To understand this serious complication, Nalle *et al.* mimicked common human transplantations in mice. Before a transplant, human patients—and the experimental mice—are irradiated to kill their own bone marrow. In the mice, however, this irradiation promoted gut “leakiness,” allowing immune-activating bacteria to enter the body. That made graft-versus-host disease worse. If medics can find a way around this sort of complication in humans, they should be on their way to safer transplants. — YN

Sci. Transl. Med. **6**, 243ra87 (2014).

so, it needs to reach throughout the body, connecting central systems to peripheral ones. Dyachuk *et al.* and Espinosa-Medina *et al.* explored how these connections are established in mice (see the Perspective by Kalchauer and Rohrer). Progenitor cells that travel along with the developing nerves can give rise to both myelin-forming Schwann cells and to parasympathetic neurons. That means the interacting nerves do not have to find each other. Instead, the beginnings of the connections are laid down as the nervous system develops. — PJH

Science, this issue p. 82, p. 87; see also p. 32

SOCIAL PSYCHOLOGY

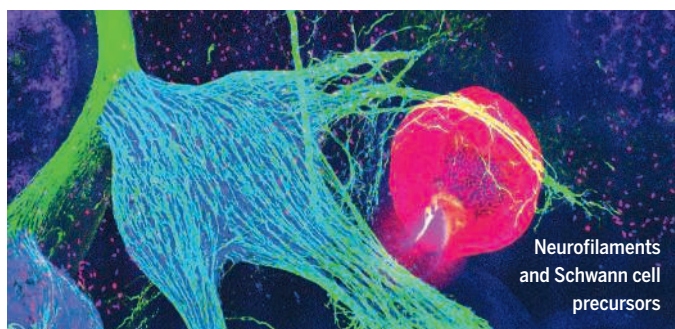
Don't leave me alone with my thoughts

Nowadays, we enjoy any number of inexpensive and readily accessible stimuli, be they books, videos, or social media. We need never be alone, with no one to talk to and nothing to do. Wilson *et al.* explored the state of being alone with one's thoughts and found that it appears to be an unpleasant experience. In fact, many of the people studied, particularly the men, chose to

NEURODEVELOPMENT

Exploiting nervous paths already traveled

The parasympathetic nervous system helps regulate the functions of many tissues and organs, including the salivary glands and the esophagus. To do



Neurofilaments and Schwann cell precursors

give themselves a mild electric shock rather than be deprived of external sensory stimuli. — GJC
Science, this issue p. 75

CANCER

Exosomes can help cancer cells hide out

Some locations in the body, such as the bone marrow, can actually help cancer cells to metastasize, or spread. Chemotherapy targets rapidly dividing cells, but cancer cells that hide out in the bone marrow proliferate slowly, which protects them from chemotherapy. Ono *et al.* found that stem cells in the bone marrow released vesicles called exosomes. Metastatic breast cancer cells took up these exosomes, which contained various factors that blocked the cancer cell proliferation. — LKF
Sci. Signal. **7**, ra63 (2014).

EXTRASOLAR PLANETS

Impolite planet ignores host's partner

Many known exoplanets (planets outside our own solar system) are hosted by binary systems that contain two stars. These planets normally circle around both of their stars. Using microlensing data taken with a worldwide network of telescopes, Gould *et al.* found a planet twice the mass of Earth that circles just one of a pair of stars. The same approach has the potential to uncover other similar star systems and help to illuminate some of the mysteries of planet formation. — MMM

Science, this issue p. 46

BILAYER GRAPHENE

Breaking down graphene degeneracy

Bilayer graphene has two layers of hexagonally arranged carbon atoms stacked on top of each other in a staggered configuration. This spatial arrangement results in degenerate electronic states: distinct states that have the same energy. Interactions

between electrons can cause the states to separate in energy, and so can external fields (see the Perspective by LeRoy and Yankowitz). Kou *et al.*, Lee *et al.*, and Maher *et al.* used three distinct experimental setups that clarify different parameter regimes of bilayer graphene. — JS

Science, this issue p. 55, p. 58, p. 61;
see also p. 31

CLIMATE CHANGE

Strong winds, upwelling, and teeming shores

Climate warming has produced stronger winds along some coasts, a result of growing differences in temperature and pressure between land and sea. These winds cause cold nutrient-rich seawater to rise to the surface, affecting climate and fueling marine productivity. Sydean *et al.* examined data from the five major world regions where upwelling is occurring. Particularly in the California, Humboldt, and Benguela upwelling systems, winds have become stronger over the past 60 years. These regions represent up to a fifth of wild marine fish catches and are hot spots of biodiversity. — HJS

Science, this issue p. 77

CELL DEATH

Life and death and quality control

When cells are subjected to too much stress, they curl up their toes and die. Lu *et al.* describe a clever strategy cells use to stay alive as long as they are not stressed for too long. The cells' quality-control machinery will activate a so-called death receptor when defective proteins accumulate within the cell, a sign of stress—but they will wait until the proteins have built up for a good long time. If stress is relieved soon enough, levels of the death receptor decay back to normal, and the cells stay alive; otherwise, R.I.P. — SMH

Science, this issue p. 98

IN OTHER JOURNALS

Edited by **Kristen Mueller**
and **Jesse Smith**

Migrating monarchs head south each fall



ZOOLOGY

Butterflies steer with magnetic compass

Each fall, eastern North American monarch butterflies migrate 4000 kilometers south to central Mexico. In daylight, the butterflies navigate by the Sun's position and their antennal circadian clocks. But under overcast skies, they rely instead on a magnetic compass. Guerra *et al.* found. The team put monarchs in a flight simulator surrounded by a magnetic coil, measuring responses to horizontal, vertical, and intensity changes in the magnetic field. Monarchs, they found, navigate north or south using the change in dip of Earth's magnetic field lines with latitude. And as in birds, the compass is light-sensitive; monarchs need ultraviolet-to-blue wavelengths to find their way. — CG

Nat. Comm. 10.1038/ncomms5164 (2014).

RISK PERCEPTION

How many words equal a number?

The probability that an event will occur can be expressed in words ("very unlikely") or in numbers ("<10%"). Budescu *et al.* show that to communicate clearly to a lay audience, it is better to use words and numbers together than words alone. They asked 10,000 adults across 25 countries to give their numerical interpretation of probability terms (*very unlikely*, *unlikely*, *likely*, or *very likely*) used in Intergovernmental Panel on Climate Change (IPCC) statements. With only words, people interpreted unlikely events to

be more likely than the IPCC intended, and vice versa. When the experimenters used both words and numerical ranges, however, the respondents estimated probabilities more accurately. — GJC

Nat. Clim. Change **4**, 508 (2014).

ARTERIAL INNERVATION

Netrin-1 helps to get the blood flowing

In moments of crisis, you might remind yourself to breathe, but generally this and other functions controlled by the autonomic sympathetic nervous system, such as swallowing or maintaining blood flow to



The whipworm
Trichuris trichurura

MICROBIAL GENETICS

Worming into host immune responses

Among the variety of parasitic worms that can infest human beings, whipworms, or trichurids, are distinct. They partially burrow into the gastrointestinal wall and immediately influence host immune responses. The worms need to persist in the gut to reproduce, which means they have to dampen their hosts' immune responses. To find the substances the worms use to modulate their hosts, Foth *et al.* sequenced the genomes of mouse- and human-infecting trichurid species and analyzed how mouse tissues responded to low-level persistent infections. The worms produced a suite of serine protease enzymes, which split intestinal glycoproteins apart, helping the worms invade the intestine. Mice responded with a muted immune response, creating an inviting environment for the worms. — CA

Nat. Genet. 10.1038/ng.3010 (2014).

the heart and central nervous system, proceed without thought. To function properly, the autonomic sympathetic nervous system requires nerves and blood vessels to coordinate with each other as they grow. Using genetic and pharmacological approaches, Brunet *et al.* find that the protein netrin-1, produced by arteries, directs sympathetic neurons to develop normally around arteries. This parallels netrin-1's role in guiding neuronal axons toward their correct targets. — BAP

J. Clin. Invest. 10.1172/JCI75181 (2014).

CANCER IMMUNOLOGY

Blocking IL-22 to stop cancer spread

Cancer metastasis, when tumors spread from their primary location, is almost always deadly, so patients need anti-metastatic therapies. Cancer stem cells (cells within the tumors that can renew themselves) may drive metastasis. Kryczek *et al.* now report that CD4⁺ T cells within human colorectal cancer tissues produce the protein interleukin-22 (IL-22), which helps to maintain colorectal cancer stem cells. IL-22 blockade slowed down colon cancer in mice, whereas IL-22 helped tumor cells express stem-cell genes

through epigenetic changes to these genes. Colon-cancer patients with these epigenetic modifications also had worse prognoses, which suggests that blocking IL-22 may provide therapeutic benefit. — KLM

Immunity 40, 772 (2014).

ECOLOGY

Casting new shade onto ecosystem thresholds

As environmental conditions change, ecosystems can suddenly enter a different and potentially unfavorable state. Researchers have captured such threshold crossings in whole-lake experiments, but it's much harder to study them experimentally in the dynamic open ocean. Thrush *et al.* cast shade on marine sandflats in New Zealand and looked at what happened to two species of bivalves. They found changes

in how the bivalves interacted, in the primary producers that they feed on, and in their physical and chemical environment. Shading causes a loss of positive feedbacks; within about 100 days, the interaction network had shifted to a new configuration. The comparatively simple experimental system helps to identify the risks of threshold crossings. — JFU

Ecology 95, 1451 (2014).

EDUCATION

A research paper in seven moves or less

Genre analysis and argumentation theory, mainstays of literature classes, rarely appear in science classrooms. To teach students how to read primary research papers efficiently, Lacum *et al.* showed students which rhetorical moves occur in research articles and how to identify them. The authors created a scientific argumentation model (SAM), which provided a detailed description of seven possible rhetorical moves scientific authors use to argue:

motive, objective, main conclusion, implication, support,

counterargument, and refutation. They then showed students how to identify these moves in a research article. A pre- and post-test that evaluated the teaching method showed a significant increase in how well students could identify these moves. — MM

CBE Life Sci. Educ. 13, 253 (2014).

ASTRONOMY

Nova seems shell-shocked after outburst

With a generous companion star, even a runty white dwarf can quickly reach explosive stature. That's probably what happened to the unusual recurring nova T Pyx, which had its sixth recorded outburst in 2011. Chomiuk *et al.* used the Swift and Suzaku satellites to observe the x-ray brightness of this system over the first few hundred days after its discovery. The high- and low-energy x-ray behavior suggest that the white dwarf ejected two shells of material in successive thermonuclear events. In the team's model, the second shell expanded 50% faster than the first, and its inevitable catch-up produced a shock responsible for the x-ray emission. The reason for this stalled secondary explosion is still unclear. — MMM

Astrophys. J. 10.1088/0004-637X/788/2/130 (2014).



Bivalves on marine sandflats in New Zealand

ALSO IN SCIENCE

Edited by Stella Hurtley

HUMAN EVOLUTION

Pleistocene people and environments

In the past few decades, hundreds of hominin fossils have been recovered from well-dated sites in East Africa. In addition, early representatives from far outside Africa have been found in Asia and Europe. Recently, discoveries at Malapa in South Africa and at Dmanisi in western Asia have brought important new fossils and archaeological residues to light. Analyses of local stratigraphy, windblown dust, sea and lake cores, and stable isotopic analyses have improved the reconstruction of ancient environments inhabited by early humans. Antón *et al.* review recent evidence and arguments about the evolution of early *Homo*, arguing that habitat instability and fragmentation imposed an important selective force. — AMS

Science, this issue p. 45

MOLECULAR KINETICS

Outliers dominate signaling at cell membrane

SOS enzymes act at cell membranes to activate Ras, a regulatory protein often overactive in cancer cells. Iversen *et al.* devised a system where they could observe the activity of individual enzymes at work. The single SOS molecules occupied stable states that varied greatly in their catalytic activity. Regulation appeared to occur by altering the time spent in active states. The overall activity of SOS was determined by just a few molecules that achieved the highest catalytic activity. The methods described should

allow further detailed kinetic analysis of this and other signaling events that occur at the cell membrane — properties that it is not possible to discern from bulk biochemical measurements. — LBR

Science, this issue p. 50

SEPARATION MEMBRANES

High-surface-area gas separation membranes

Membranes for gas separation require a combination of high surface area and selective transport pathways. Brown *et al.* present a potentially scalable route for making high-quality gas separation membranes in a high-surface-area configuration. Using two different solvents flowing in opposite directions, a metal-organic framework material was selectively deposited within hollow polymer fibers. The membranes showed high-performance separation capabilities when tested with mixtures of hydrocarbon gases. — MSL

Science, this issue p. 72

ACTIVE GALAXIES

Gas jets block extragalactic x-rays

Supermassive black holes at the heart of active galaxies produce powerful gas outflows. NGC 5548 is one such source known to sustain a persistent outflow of ionized gas. However, its associated x-ray and ultraviolet (UV) emission seem to have been suppressed in recent years. Kaastra *et al.* conducted a multiwavelength monitoring campaign throughout 2013 to characterize the system's behavior. They suggest that an

additional faster jet component has been launching clumps of gas that obscure both the x-ray and UV radiation. The timing of this phenomenon indicates a source only a few light-days away from the nucleus. This proximity suggests that the outflow could be associated with a wind from the supermassive black hole's accretion disk. Even more powerful outflows could also influence their host galaxies, and this finding demonstrates how that feedback might work. — MMM

Science, this issue p. 64

C-H BOND ACTIVATION

Carbon-carbon bonds without byproducts

Environmental and cost concerns are spurring development of chemical methods that minimize byproduct formation. In this vein, Mo and Dong present a catalyst that inserts olefins such as ethylene directly into the C-H bonds of ketones. Traditional methods to form such products rely on the preliminary reaction of the ketone with a base, followed by subsequent reaction with an alkyl halide. The authors used a ligand that simultaneously activates the ketone and guides the catalytic rhodium to the right location. This approach removes the need for the other reagents and eliminates the associated halide salt byproducts. — JSY

Science, this issue p. 68

EARTHQUAKE DYNAMICS

Seismic noise reveals volcanic plumbing

Monitoring the way in which

seismic noise passes through Earth's crust after a large earthquake can clarify how volcanoes erupt. Japan has the highest-density seismic network in the world. Brenguier *et al.* observed reductions in seismic velocity below volcanic regions of Japan from before, to the weeks and months after the 2011 Tohoku-Oki earthquake (see the Perspective by Prejean and Haney). This indicates that pressurized fluids below volcanoes can weaken in response to dynamic stress perturbations. — NW

Science, this issue p. 80; see also p. 39

PLANT-FUNGAL ECOLOGY

It takes two to tango in restricted environments

Despite being unrelated, free-living algae and fungi can learn to help one another out. Hom and Murray raised the green alga *Chlamydomonas reinhardtii* in CO₂-restricted environments in the presence of the yeast *Saccharomyces cerevisiae* (see the Perspective by Aanen and Bisseling). The experimental setup forced the two species to depend on one another for the metabolic production of CO₂, which is provided by the yeast as it consumes glucose and is needed by the alga, and ammonia, which conversely can be made from nitrite by the alga and then used by the yeast. This dependence was seen under a broad range of environmental conditions. Similar tests between other *Chlamydomonas* and fungal species revealed the ability to create a phylogenetically broad range of mutualisms. — LMZ

Science, this issue p. 94; see also p. 29

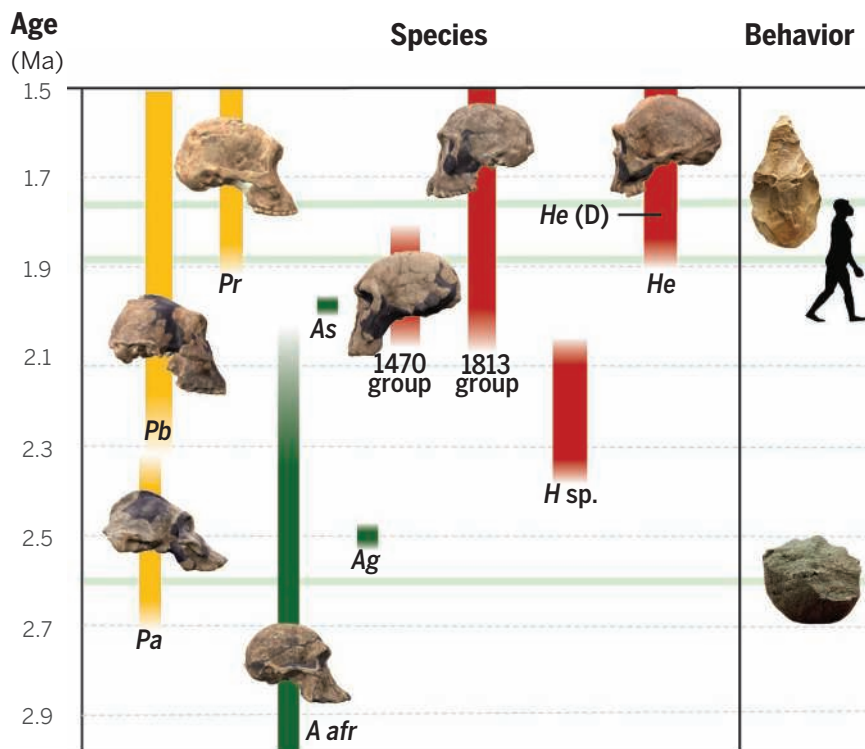
HUMAN EVOLUTION

Evolution of early *Homo*: An integrated biological perspective

Susan C. Antón,¹ Richard Potts,² Leslie C. Aiello³

BACKGROUND: Until recently, the evolution of the genus *Homo* has been interpreted in the context of the onset of African aridity and the expansion of open grasslands. *Homo erectus* was considered to be a bona fide member of the genus *Homo*, but opinions diverged on the generic status of earlier, more fragmentary fossils traditionally attributed to *Homo habilis* and *Homo rudolfensis*. Arguments about generic status of these taxa rested on inferred similarities and differences in adaptive plateau.

However, there was near-universal agreement that the open-country suite of features inferred for *Homo erectus* had evolved together and provided the adaptations for dispersal beyond Africa. These features foreshadowed those of more recent *Homo sapiens* and included large, linear bodies, elongated legs, large brain sizes, reduced sexual dimorphism, increased carnivory, and unique life history traits (e.g., extended ontogeny and longevity) as well as toolmaking and increased social cooperation.



Hominin evolution from 3.0 to 1.5 Ma. (Species) Currently known species temporal ranges for Pa, *Paranthropus aethiopicus*; Pb, *P. boisei*; Pr, *P. robustus*; A afr, *Australopithecus africanus*; Ag, *A. garhi*; As, *A. sediba*; H sp., early *Homo* >2.1 million years ago (Ma); 1470 group and 1813 group representing a new interpretation of the traditionally recognized *H. habilis* and *H. rudolfensis*; and He, *H. erectus*. He (D) indicates *H. erectus* from Dmanisi. (Behavior) Icons indicate from the bottom the first appearance of stone tools (the Oldowan technology) at ~2.6 Ma, the dispersal of *Homo* to Eurasia at ~1.85 Ma, and the appearance of the Acheulean technology at ~1.76 Ma. The number of contemporaneous hominin taxa during this period reflects different strategies of adaptation to habitat variability. The cultural milestones do not correlate with the known first appearances of any of the currently recognized *Homo* taxa.

ADVANCES: Over the past decade, new fossil discoveries and new lines of interpretation have substantially altered this interpretation. New environmental data sets suggest that *Homo* evolved against a background of long periods of habitat unpredictability that were superimposed on the underlying aridity trend. New fossils support the presence of multiple groups of early *Homo* that overlap in body, brain, and tooth size and challenge the traditional interpretation of *H. habilis*

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and *H. rudolfensis* as representing small and large morphs, respectively. Because of a fragmentary and distorted type specimen for *H. habilis* two informal morphs are proposed, the 1813 group and the 1470 group, that are distinguished on the basis of facial anatomy but do not contain the same constituent fossils as the more formally designated species of early *Homo*. Furthermore, traits once thought to define early *Homo*, particularly *H. erectus*, did not arise as a single package. Some features once considered characteristic of *Homo* are found in *Australopithecus* (e.g., long hind limbs), whereas others do not occur until much later in time (e.g., narrow pelvis and extended ontogeny). When integrated with our understanding of the biology of living humans and other mammals, the fossil and archaeological record of early *Homo* suggests that key factors to the success and expansion of the genus rested on dietary flexibility in unpredictable environments, which, along with cooperative breeding and flexibility in development, allowed range expansion and reduced mortality risks.

OUTLOOK: Although more fossils and archaeological finds will continue to enhance our understanding of the evolution of early *Homo*, the comparative biology of mammals (including humans) will continue to provide valuable frameworks for the interpretation of existing material. This comparative context enables us to formulate and test robust models of the relationships between energetics, life history, brain and body size, diet, mortality, and resource variability and thereby yield a deeper understanding of human evolution. ■

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REVIEW

HUMAN EVOLUTION

Evolution of early *Homo*: An integrated biological perspective

Susan C. Antón,¹ Richard Potts,² Leslie C. Aiello³

Integration of evidence over the past decade has revised understandings about the major adaptations underlying the origin and early evolution of the genus *Homo*. Many features associated with *Homo sapiens*, including our large linear bodies, elongated hind limbs, large energy-expensive brains, reduced sexual dimorphism, increased carnivory, and unique life history traits, were once thought to have evolved near the origin of the genus in response to heightened aridity and open habitats in Africa. However, recent analyses of fossil, archaeological, and environmental data indicate that such traits did not arise as a single package. Instead, some arose substantially earlier and some later than previously thought. From ~2.5 to 1.5 million years ago, three lineages of early *Homo* evolved in a context of habitat instability and fragmentation on seasonal, intergenerational, and evolutionary time scales. These contexts gave a selective advantage to traits, such as dietary flexibility and larger body size, that facilitated survival in shifting environments.

The evolution of the genus *Homo* has long been linked to the onset of African aridity, and the evolution of key features such as increased carnivory, brain enlargement, long-distance mobility, and prolonged life history. These features have been explained as a response to the progressive expansion of open, grassland habitats (1, 2). However, new environmental data challenge this interpretation, and archaeological research has identified behaviors in early toolmakers that aided flexible responses to dynamic environments (3, 4). Furthermore, comparative studies of mammalian development, energetics, ecology, and behavior offer new interpretive models. In this context, new fossils have also expanded the known range of morphological variation, raising questions about the number of species of early *Homo* and the distinction between inter- and intraspecific adaptations (5–10).

The East African fossil record continues to command much attention because of a unique combination of factors. The history of East African rift volcanism enables precise geochronological analyses through long stratigraphic sequences rich in fossil and archaeological remains. The temporal sequence of morphological and behavioral innovations in early *Homo* is thus more finely resolved in East Africa than elsewhere. Environmental indicators can also be measured in lengthy stratigraphic order, enabling researchers to assess climate and habitat dynamics at a variety of time

scales rather than relying on more limited environmental snapshots or broadly time-averaged portraits of the environment. Uncertainties over stratigraphic correlation and dating have arisen that directly affect an understanding of early *Homo*, yet East African rift basins typically offer opportunities to resolve the geological debates [e.g., (11, 12)]. Beyond this region, important recent finds pertinent to the evolution of *Homo* have been made at Malapa, South Africa (6, 7, 9, 13), and Dmanisi, Georgia (8), which expand how hominin morphological variation and the dispersal of early *Homo* beyond Africa are understood. This review begins with a focus on morphological variation and environmental dynamics because these topics have strongly affected analyses of the adaptive shifts distinctive to early *Homo* (Fig. 1).

Who was early *Homo*?

Throughout the 20th century, the definition of *Homo* was expanded to accommodate fossil specimens increasingly remote from *Homo sapiens* in both time and morphology [e.g., (14, 15)]. Landmarks include collapsing multiple genera into *Homo erectus* in the 1940s, naming *Homo habilis* in 1964, and establishing *Homo rudolfensis* in 1986 (14, 16, 17). However, the status of pre-*erectus* *Homo* has always been controversial, and by the late 1990s the perceived similarities between fossil remains of *Australopithecus* (especially *A. afarensis*, e.g., A.L. 288-1; “Lucy”) and non-*erectus* early *Homo* (e.g., fossil specimens KNM-ER 1470 and 1813) led some to reclassify both *H. habilis* and *H. rudolfensis* as *Australopithecus* (18, 19) and more recently to suggest that they might belong to a new, unspecified genus (20). Alternatively, anatomical variation within early *H. erectus* at Dmanisi has been used to argue not only for the inclusion of these specimens in early *Homo* but for the inclusion of all early *Homo* in a single species, *H. erectus* (8).

The argument for excluding non-*erectus* *Homo* from the genus rested heavily on differences in adaptive plateau, particularly dietary adaptation, and locomotor efficiency inferred from aspects of postcranial anatomy. However, for all hominins subsequent to ~3.5 million years ago (Ma) new isotopic studies identify a diverse diet incorporating a broad range of plants using the C₃ and C₄ photosynthetic pathways (21, 22) (Fig. 1C). Furthermore, large-bodied finds of *Australopithecus* (23) and small-bodied *Homo* show no difference in hind limb proportions or inferred bipedal efficiency; this is because locomotor efficiency in walking and running is a function of leg length, which is allometrically related to body size (24). Similarly, the *A. afarensis* foot possessed close-packed arches, another sign of bipedal adequacy (25, 26). Although there may have been multiple modes of bipedality among the early hominins, long legs and efficient bipedal locomotion were in place well before the origin of the genus *Homo* and cannot necessarily be used to distinguish among genera or species. Regardless of the taxonomy of early *Homo* or morphological differences between species, recent fossil finds and new analytical techniques suggest that all early *Homo* differ from *Australopithecus* in having larger average body and brain sizes (Table 1).

Given these observations, what is the evidence for distinct morphological groups in the fossil record of *Homo* before and contemporaneous with *H. erectus*? The earliest fossils assignable to *Homo* are fragmentary and identified by reduced tooth and jaw size and the shape and reorganization of craniofacial morphology (supplementary materials) (5, 10, 27–32). Among the oldest and most complete are likely to be the A.L. 666 maxilla from Ethiopia (~2.33 Ma), which has some affinities to hominins traditionally called *H. habilis* (33), and the UR-501 mandible [2.5 to 1.9 Ma; (29, 34)] from Malawi, which is more robust and similar to mandibles (i.e., KNM-ER 1802) often included in *H. rudolfensis*. A few South African fossils over 2.1 Ma also may be attributed to early *Homo*, although they are usually considered *Australopithecus* (35–39). Brain size and postcranial anatomy are largely unknown for this period.

There are many more early *Homo* specimens between 2.1 and 1.5 Ma, as well as new contenders for relatives of *Homo*. The recently discovered *Australopithecus sediba* (~1.98 Ma) from Malapa, South Africa, is argued to possess a unique relationship to the origin of *Homo* because of a number of *Homo*-like features of its cranial and postcranial anatomy, particularly a reduction in dental size and aspects of its pelvis and lower thorax, although it differs from *Homo* in cranial capacity, facial shape, and aspects of the postcranial skeleton (6, 7, 13). In addition to *A. sediba*, at least one group of early *Homo* is likely present in South Africa, based on dental anatomy (35, 39), although the highly fragmentary nature of the remains make associations with East African forms speculative.

East African non-*erectus* *Homo* from this period has been assigned previously to either *H. habilis*

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or *H. rudolfensis*, which were often considered to represent small- and large-brained (and -bodied) species, respectively (19, 32, 40, 41). New fossils from Lake Turkana, Kenya (KNM-ER 60000 and 62000), suggest multiple species of non-*erectus* *Homo* just after 2.1 Ma but show that the two species cannot be distinguished on the basis of cranial size (5). The new Kenyan fossils suggest that palate and mandibular shape, especially the relative position of the anterior dentition, differentiate among the two better known groups of early *Homo*; yet taphonomic damage to the OH 7 mandible, the type specimen for *H. habilis*, and the fact that size may no longer be a distinguishing feature of different species of early *Homo* preclude an easy answer to the attribution of the type. This poses nomenclatural problems because it is unclear to which group, if either, the nomen *H. habilis* applies (5). We therefore recommend informally calling the morphological groups of early non-*erectus* *Homo* after their most iconic specimens (10). Thus, the 1470 group (2.09 to 1.78 Ma, table S1) is named for KNM-ER 1470 and is distinguished particularly by its short and flat anterior

dental arcade (with a short premolar row and flat anterior tooth row) and by a relatively tall, flat face. The 1813 group (2.09 to 1.44 Ma) has a more primitive face with a round and more projecting anterior palate and is named for KNM-ER 1813.

We emphasize that these groups do not comprise the same fossils as previously attributed to *H. rudolfensis* and *H. habilis*. Fossils with large teeth but primitive arcade structures (such as KNM-ER 1802) are definitively excluded from the 1470 group, and large fossils (such as KNM-ER 1590) that were once grouped with KNM-ER 1470 on the basis of size alone are now unaffiliated because they do not preserve critical anatomical areas. As a result of these reassignments, both the 1813 and 1470 groups exhibit considerable and overlapping size variation. In particular, molar size, facial size (but not shape), and very likely endocranial and body size cannot be used to distinguish the 1813 and 1470 groups (Box 1, supplementary text, and table S2) as they once were used to distinguish *H. habilis* and *H. rudolfensis*.

In contrast with these groups, but partly overlapping them in time, is early African *H. erectus*

(~1.89 to 0.90 Ma), which has been traditionally distinguished from non-*erectus* early *Homo* on the basis of dental anatomy, craniofacial morphology, and average cranial and body size [e.g., (10, 42, 43); see Box 1]. Cranial fossils KNM-ER 42700 and KNM-OG 45500 substantially extend the lower end of the size range, overlapping with non-*erectus* early *Homo* (32, 44). Postcranial fossils from Gona, Ethiopia (45), and reevaluation of the KNM-WT 15000 skeleton (46) suggest small-size individuals, as well as a less-linear body form than previously thought. Early *H. erectus* is best known from East Africa, although there are hints of its presence in South Africa (39, 44, 47–51). Shortly after its appearance in Africa, *H. erectus* is also found at Dmanisi, Georgia (52); Java, Indonesia (53, 54); and possibly Yuanmou, China (55); providing evidence of range expansion across Asia. Individual and regional variations exist, including substantial variation in size, especially between the Georgian and some African fossils (32, 44, 56). However, there is growing consensus that these represent regional morphs of a single species (42, 57, 58).

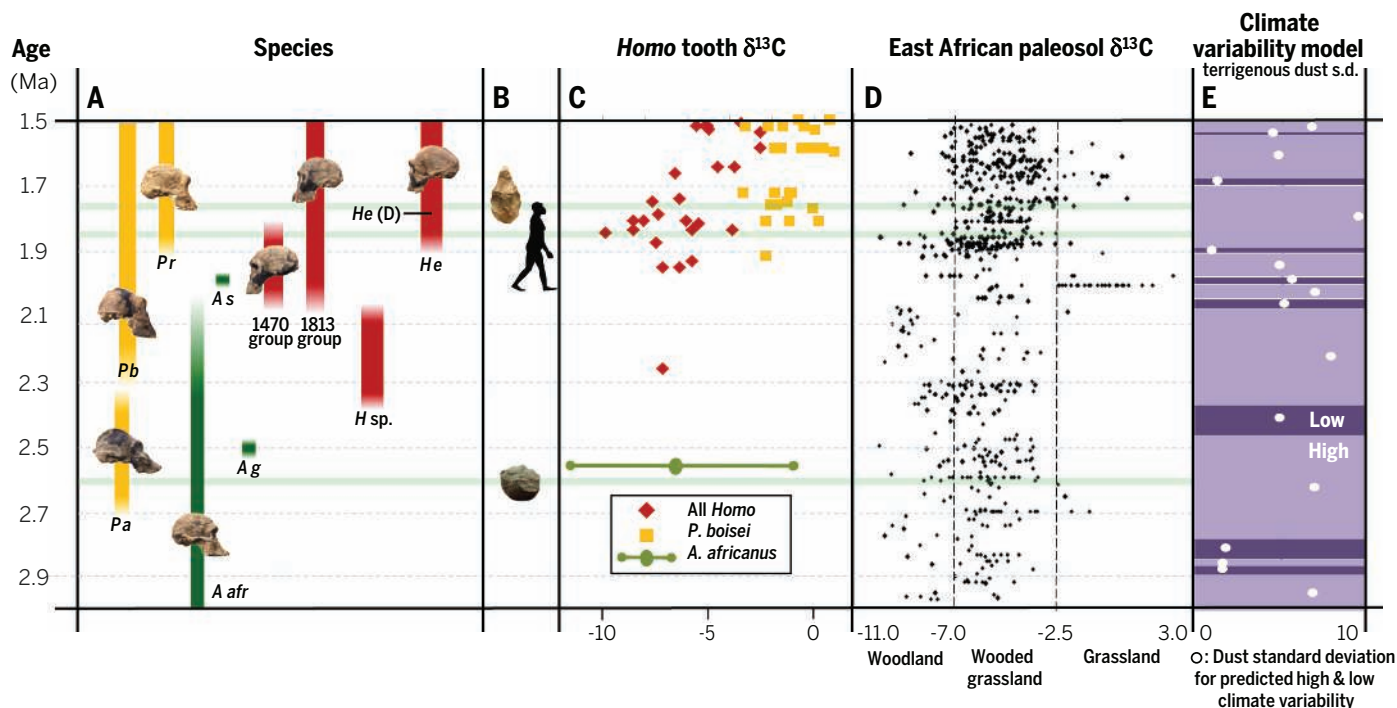


Fig. 1. Hominin evolution, diet, landscape vegetation, and climate dynamics from 3.0 to 1.5 Ma. (A) Currently known species temporal ranges for Pa, *Paranthropus aethiopicus*; Pb, *P. boisei*; Pr, *P. robustus*; Aafr, *Australopithecus africanus*; Ag, *A. garhi*; As, *A. sediba*; H sp., early *Homo* > 2.1 Ma; 1470 and 1813 groups, see text for definitions (traditionally classified as *H. rudolfensis* and *H. habilis*, respectively); and He, *H. erectus*. The temporal position of Dmanisi *H. erectus*, He (D), is indicated. (B) Icons representing the first appearance of (from bottom) Oldowan technology (~2.6 Ma), *Homo* dispersal to Eurasia (~1.85 Ma), and Acheulean technology (~1.76 Ma). Horizontal pale green lines mark these times across (A) to (D). (C) *Homo* tooth $\delta^{13}\text{C}$. Carbon isotopic values measured on tooth enamel of East African specimens assigned to *Homo* and *P. boisei* (21); the mean and range of dental $\delta^{13}\text{C}$ for *A. africanus* is also shown (22). (D) East African paleosol $\delta^{13}\text{C}$: compilation of $\delta^{13}\text{C}$ values for East African fossil soil carbonates [data compiled in (74)]. Values range from those typical of woodland (40 to 80% woody cover)

to wooded grassland (10 to 40% woody cover) to grassland (0 to 10% woody cover). Woody cover estimates based on (2). (E) Climate variability. Alternating intervals of high (lighter color bands) and low (darker color bands) climate variability based on predicted insolation resulting from the modulation of orbital precession by eccentricity, where low variability is defined by eccentricity $e \leq 0.0145$, (i.e., 1 SD below mean e for the past 5 million years) (67). White circles show the standard deviations for terrigenous dust flux values at Ocean Drilling Project sites 721 and 722, western Arabian Sea (64, 69). Change between eolian dust standard deviations (adjacent white circles) follows the predicted direction between alternating high (larger SD, further to the right) and low (smaller SD, further to the left) climate variability for 13 of the 16 variability transitions. For example, the large SD in the two predicted high climate variability intervals, 2.79 to 2.47 and 2.37 to 2.08 Ma, is further to the right of the plot than is the intervening small SD in the predicted low-variability interval 2.47 to 2.37 Ma.

Table 1. Comparative brain and body size of *Australopithecus* and *Homo* based on the most complete specimens in each group. See Box 1 for a discussion of how body and brain size ranges may change based on the size of more fragmentary remains in each assemblage. The 1470 and 1813 groups in particular are skewed to larger and smaller sizes, respectively, by considering only their more complete members. Individual data points included in these species' means can be found in table S2. Sources for endocranial capacity data are as follows: *A. sediba* (6); *A. africanus* (127); *A. afarensis* (128); *Homo*, as indicated in table S2 of this paper. The apparent difference in cranial capacity between the 1470 and 1813 groups is due to the fact that only a single large cranium, KNM-ER 1470, contributes to the capacity for that group. Dental measurement definitions and most data are from (41); *A. afarensis*, from (129); *A. sediba*, as above; newer *Homo* data not in (41)

follow (5, 32, 130). I^1 , upper incisor 1; I_1 , lower incisor 1. Body mass estimates from orbital dimensions are from (131, 132). The values presented are the range of individual predicted values; the mean of individual predicted values rounded to whole values; and the total range of the 95% confidence intervals for all individual values. The Kappelman values are predicted from orbital area. The Aiello and Wood values are predicted from orbital height dimensions and hominoid predictive equations. The apparent difference in body mass between 1470 and 1813 groups is because only a single large cranium, KNM-ER 1470, contributes to the body mass estimate for that group. Post-cranial body mass estimates follow (24) and table S2 of this paper. The East Africa unattributed non-*erectus* *Homo* group includes *H. habilis*. CV, coefficient of variation; CI, confidence interval; dash entries, not applicable or no fossils or data available.

	South Africa <i>A. sediba</i>	South Africa <i>A. africanus</i>	East Africa <i>A. afarensis</i>	East Africa 1470 group	East Africa 1813 group	East Africa unattributed non- <i>erectus</i> <i>Homo</i>	East Africa All non- <i>erectus</i> <i>Homo</i>	South Africa <i>H. aff.</i> <i>erectus</i>	East Africa/ Georgia early <i>H. erectus</i>
<i>Brain Size (cm³)</i>									
Range	420	385–571	400–550	750*	510–660*	630–680	510–750	–	546–1067 (with Dmanisi) 690–1067 (East Africa)
Mean	–	459	478	–	586	650	629	–	787 (with Dmanisi) 863 (East Africa)
CV	–	15.9	15.7	–	10.5	5.4	12.2	–	20.2 (with Dmanisi) 15.9 (East Africa)
<i>Dental Size</i>									
<i>I¹ labio-lingual (mm)</i>									
Range	6.9	6.5–8.7	7.1–9.7	–	8.0	7.2–8.2	7.2–8.2	–	7.6–9.4 (with Dmanisi) 8.2–9.4 (East Africa)
Mean	–	8.0	8.4	–	–	7.7	7.8	–	8.4 (with Dmanisi) 8.8 (East Africa)
CV	–	12.5	8.5	–	–	6.5	5.6	–	10.9 (with Dmanisi) 9.6 (East Africa)
<i>I₁ labio-lingual (mm)</i>									
Range	–	5.9–6.9	6.9–7.7	5.4	–	6.6–7	5.4–7	–	6.0–6.9 (East Africa)
Mean	–	6.5	7.4	–	–	6.8	6.3	–	6.45 (East Africa)
CV	–	8.1	4	–	–	4.2	13.1	–	9.9 (East Africa)
<i>I² labio-lingual (mm)</i>									
Range	5.1	6.4–7.2	6.6–8.2	–	6.1–7.3	5.8–8.2	5.8–8.2	–	6.9–8.5 (with Dmanisi) 8.1–8.5 (East Africa)
Mean	–	6.9	7.5	–	6.7	7.2	7.1	–	7.8 (with Dmanisi) 8.3 (East Africa)
CV	–	4.9	7.5	–	12.7	15.2	13.8	–	10.6 (with Dmanisi) 3.4 (East Africa)
<i>I₂ labio-lingual (mm)</i>									
Range	–	6.9–8.2	6.7–8.8	6.6	–	7.4–7.6	6.6–7.6	–	7.0–8.3 (with Dmanisi) 7.0–8.3 (East Africa)
Mean	–	7.7	8.0	–	–	7.5	7.2	–	7.4 (with Dmanisi) 7.4 (East Africa)
CV	–	8.6	9.6	–	–	1.9	7.3	–	8.2 (with Dmanisi) 10.1 (East Africa)
<i>M¹ area (mm²)</i>									
Range	1548	1620–2030	–	1850	1560–1790	2010–2090	1560–2090	–	1490–1770 (with Dmanisi) 1490–1770 (East Africa)
Mean	–	1795	–	–	1687	2050	1798	–	1630 (with Dmanisi) 1630 (East Africa)
CV	–	8.5	–	–	5.6	2.8	10.1	–	7.8 (with Dmanisi) 12.1 (East Africa)
<i>M₁ area (mm²)</i>									
Range	1310–1450	1470–2070	–	1460–1835	1500	1410–1950	1410–1950	–	1330–1780 (with Dmanisi) 1330–1790 (East Africa)

Continued on next page

	South Africa <i>A. sediba</i>	South Africa <i>A. africanus</i>	East Africa <i>A. afarensis</i>	East Africa 1470 group	East Africa 1813 group	East Africa unattributed non- <i>erectus</i> <i>Homo</i>	East Africa All non- <i>erectus</i> <i>Homo</i>	South Africa <i>H. aff.</i> <i>erectus</i>	East Africa/ Georgia early <i>H. erectus</i>
Mean	1380	1731	–	1683	–	1630	1631	–	1533 (with Dmanisi) 1534 (East Africa)
CV	7.2	11.9	–	11.7	–	14.2	13.2	–	11.3 (with Dmanisi) 13 (East Africa)
<i>M</i> ² area (mm ²)									
Range	1767	1960–2700	–	2016	1640–1890	1940–2570	1640–2570	–	1500–1860 (with Dmanisi) 1500–1860 (East Africa)
Mean	–	2218	–	–	1797	2177	1933	–	1622 (with Dmanisi) 1640 (East Africa)
CV	–	11.7	–	–	5.6	15.8	13.1	–	8.7 (with Dmanisi) 11.8 (East Africa)
<i>M</i> ₂ area (mm ²)									
Range	1720–1858	–	–	1790–2100	1680–1840	1650–2730	1650–2730	–	1380–1990 (with Dmanisi) 1490–1770 (East Africa)
Mean	1789	–	–	1945	1760	2128	2043	–	1573 (with Dmanisi) 1660 (East Africa)
CV	5.5	–	–	11.3	6.4	16.2	15.8	–	14.1 (with Dmanisi) 13.7 (East Africa)
Body Size									
Body mass from orbits (kg)									
Predicted value range									
Kappelman	–	27–28	–	46	30–35	–	30–46	–	57–59 (East Africa)
Aiello	–	22–29	–	51	31–36	–	31–51	–	58–65 (East Africa)
Mean of predicted									
Kappelman	–	27	–	–	32	–	37	–	58 (East Africa)
Aiello and Wood	–	25	–	–	34	–	39	–	61 (East Africa)
CI range									
Kappelman	–	19–40	–	34–70	21–50	–	21–70	–	40–86 (East Africa)
Aiello and Wood	–	19–36	–	43–63	22–42	–	22–63	–	48–82 (East Africa)
Body mass from postcrania (kg)									
Range	32–36	23–45	27–50	–	–	31–57	31–57	30–58	40–68 (with Dmanisi) 40–68 (East Africa)
Mean	33	34	40	–	–	44	44	47	52 (with Dmanisi) 55 (East Africa)
CV	9	18.7	20.2	–	–	31	31.5	32	19.3 (with Dmanisi) 18.8 (East Africa)
Femur length (mm)									
Range	–	276–434	281–382	–	–	396–401	315–401	–	429–485 (East Africa)
Mean	–	355	346	–	315	398	398	–	450.5 (East Africa)
CV	–	–	16.3	–	–	1	13	–	5.8 (East Africa)

*Listed ranges for brain and body size reflect only a limited number of fossils with sufficient preservation for estimates but do not reflect the size diversity in the groups as known from fragmentary remains. Thus, because the small face KNM-ER 62000 is about 80% the size of KNM-ER 1470, we conservatively estimate that the lower range of brain size for this group may be as little as 560 cm³; and, because the largest capacity of the 1813 group (from OH 13) is from an individual with a palate just 85% the size of the large OH 65, the upper value in that group may be around 775 cm³.

New fossils from Dmanisi, Georgia (~1.8 Ma), exhibit a range of variation that is argued to encompass not only that seen in early African *H. erectus* but also that of all other early African *Homo* as well (8). The five Dmanisi crania, some with associated postcranial remains, exhibit derived characters of *H. erectus* but also retain some primitive characteristics, including small brain sizes (538 to 750 cm³), suggesting they are part of an early dispersal of that species. A global analysis of craniofacial size and shape was used to argue that this expanded range of variation encompassed all three morphs of African *Homo* (i.e., 1470 and

1813 groups and *H. erectus*) (8) and thus that only a single lineage of *Homo* existed and perhaps even originated at Dmanisi. However, this metric analysis of overall cranial shape misses the specific characters, as described above (supplementary materials and Box 1), that distinguish these groups and cannot, therefore, be used to disprove their existence (20, 59). Although we concur that a more robust fossil record is surely necessary, we conclude that three distinct lineages of early *Homo* in Africa remains the most compelling hypothesis.

Thus, the East African fossil record provides evidence of at least three partly contemporary

species of *Homo* from ~2 to 1.5 Ma, all of which exhibit, on average, larger brains and bodies than *Australopithecus* (Table 1). Non-*erectus* early *Homo* (i.e., both 1470 and 1813 groups) is about 30% bigger in brain and 10% bigger in body size than *Australopithecus*. Early African and Georgian *H. erectus* together are about 40% bigger in brain and 25% in body size than *Australopithecus*. Early *H. erectus* is 20% bigger in brain and 15% in body than the combined 1470 and 1813 groups. Importantly, ranges of variation overlap substantially, and there is also no discernible difference in sexual dimorphism

Box 1. Anatomical features of early *Homo* groups

Fragmentary fossils provide the hard evidence for the anatomy and variation within early *Homo*. Making inferences regarding the number of groups is a nontrivial exercise that relies on careful assessments of anatomical similarity and the recognition of differences in morphological traits and their patterning across fossil assemblages. The main anatomical features of the three groups of early *Homo* and their fossil group members are summarized below, and in the supplementary text we provide additional discussion of the fossils that compose these groups and those that we currently cannot assign to a group because of missing evidence.

(A) The 1470 group is defined by the derived shape of the face, which is relatively tall and flat with the incisor/canine row squared off and the upper third premolar forming the corner of the anterior palate. Lower incisors are narrow. Premolars are mesiodistally narrow and molars are large but just slightly larger than average for all early *Homo*. There is no third molar reduction. The vault is rounded and lacks a posttoral/supratotal gutter. The posterior mandible (bigonial/bicondylar breadths) is wide relative to arcade breadth, and the corpus is relatively tall. Only the largest specimen, KNM-ER 1470, allows actual estimates of brain size (750 cm³) or body size (43 to 63 kg, from the orbit), but differences in size between KNM-ER 1470 and the more fragmentary KNM-ER 62000 suggest that the lower range of brain and body size is substantially less. Given that facial dimensions of KNM-ER 62000 are between 75 and 80% of those of KNM-ER 1470, we cautiously suggest ranges of 560 to 750 cm³ and 35 to 50 kg for this group.

Key members: cranium KNM-ER 1470; partial face 62000; mandibles KNM-ER 1482 and 60000. No postcranial remains are affiliated with this group.

Important exclusions: Mandibles KNM-ER 1802 and Uraha 501 are definitively excluded from this group on the basis of arcade shape and a mismatch with the palate of KNM-ER 62000 (5). Cranial specimens once included with the group on the basis of large cranial and dental size, especially KNM-ER 1590, have insufficient preservation in key areas (mandible and face) to allow an assessment of palate or mandibular shape. These certain and probable exclusions remove evidence for extremely large molar size in this group.

(B) The 1813 group presents a more primitive facial architecture with a rounded anterior palate and more parallel and narrow posterior tooth rows. Lower incisors are broader than in the 1470 group (uppers are unknown for the 1470 group). Molars are about the size of or slightly smaller than the 1470 group; however, if the large-molared KNM-ER 1802 is included, as we suspect it should be, these differences disappear entirely. There is no third molar reduction. Mandibular height and width are similar; rami are essentially unknown. The vault is rounded with some posterior occipital cresting in some individuals (e.g., KNM-ER 1805). Brain size estimates from the best preserved of these yield a range of 510 to 660 cm³; however, because the OH 65 palate (which does not preserve the cranial vault) is about 15% bigger than that of OH 13 (which is associated with a vault of 660 cm³), we cautiously suggest that the upper range may increase to as much as 775 cm³. Cross-sectional data indicate the group had relatively strong upper limbs compared with lower limb strength, suggesting a sustained arboreal component (perhaps related to nesting) in addition to their terrestrial locomotor repertoire (134). Body size estimates are available from only the smallest specimens (e.g., KNM-ER 1813 from orbital dimensions and OH 62 from the postcranial skeleton) and suggest ranges of 30 to 35 kg. The size of OH 65 relative to KNM-ER 1813 suggests that the upper range of these should be extended to at least 42 kg.

Key members: crania KNM-ER 1813, OH 24; partial crania and mandible KNM-ER 1805, OH 13; palate OH 65; fragmentary cranial and postcranial KNM-ER 3735, OH 62.

Likely members: Mandible KNM-ER 1802 and Uraha 501 are definitively excluded from the 1470-group and are consistent with palate shape in the 1813 group. However, because they are more robust and because OH 7 remains unaffiliated, leaving open the possibility of a third group of non-*erectus* *Homo*, we suggest they are likely but not certain members of the 1813 group.

Important unknowns: OH 7 cannot be definitively affiliated with any group. Although the specimen retains a mandible and dentition, extensive postmortem deformation and distortion to the mandibular symphysis and body leave the relationships among and between the anterior and posterior tooth rows unresolved. It is thus currently impossible to assess the fossil for the key features of arcade shape and orientation that distinguish the 1813 and 1470 groups.

(C) Early *H. erectus* is represented by a greater number of fossil crania and a larger geographic distribution of samples. The face and dental arcade lacks the derived anatomy of the 1470 group arcade, being in some ways more similar to the 1813 group with the rounded anterior palate and large incisors. However, unlike this group posterior arcade shape is more derived, with broader more parabolic tooth rows and third molar crown reduction. The canines (especially roots) and premolars are also reduced relative to the condition in the 1813 group (but the premolars are not narrow as in 1470 group). The mandibular body is more gracile than in the 1813 group. The vault is rounded but presents a variable series of superstructures (some size-related) including supraorbital tori and posttoral/supratotal gutter, bregmatic and sagittal keels, and angular and occipital tori; however, cresting is not seen. The petrous temporal is angulated around the glenoid fossa. Shaft cross-sectional data suggest relatively less strong upper limb to lower limb development, which has been suggested to reflect greater terrestriality than in the 1813 group (133). Between 1.9 and 1.5 Ma, substantial regional population variation in size exists, but taken together the brain size estimates from the best preserved of these yield a range of 546 to 1067 cm³, and postcranial body mass estimates suggest ranges of 40 to 68 kg.

Key members from Africa and Georgia: crania and calvaria KNM-ER 3733, KNM-ER 3883, and KNM-ER 42700, OH 9, and Dmanisi 2280; crania and associated mandibles Dmanisi 2282/211, 2700/2735, and 3444/3900; crania and associated postcrania KNM-ER 1808 and 15000.

between species or genera (24, 60–62) (Tables 2 and 3).

The Dmanisi fossils, as well as *A. sediba*, highlight the importance and the difficulties of recognizing and distinguishing two important aspects of variation in early *Homo*: variation within and between species. The Dmanisi remains, along with small-sized remains from East Africa, have expanded the range of size variation within *H. erectus*, highlighted the notion of population-level variation within that taxon, and blurred at

least the size distinctions among morphological groups of early *Homo*. The mosaic of features in *A. sediba* (~1.98 Ma) and variation in the Dmanisi *H. erectus* sample (~1.8 Ma), both of which are contemporaneous with the three African groups, suggest that the early diversification of *Homo* was a period of morphological experimentation. The potential malleability of developmental processes and the role of vicariance and hybridization in evolving and testing reproductive and correlated morphological distinctions remain im-

portant sources of uncertainty, although ones potentially ripe for future evaluation through integration of data from extant biological forms.

Environmental instability as an evolutionary paradigm

The intra- and intertaxon diversity observed in early hominins cannot be understood apart from its environmental context. A long-standing view is that human evolution was linked to the onset of global cooling, progressive African aridity,

Table 2. Differences between *Australopithecus* and *Homo* and within early *Homo* based on empirical fossil and archaeological evidence.

These differences may relate to life history inferences made in Table 3. *A. afarensis* is used as a conservative comparator because it has the largest average brain (128) and body sizes (24) of the more complete *Australopithecus* species. Non-*erectus* early *Homo* includes specimens in the 1470 and 1813 groups and non-*erectus* early *Homo* fossils not yet attributed to either of the former groups. Early *H. erectus* values are the combined means for Dmanisi and early African *H. erectus* as a conservative comparison with *A. afarensis*, followed by the early African *H. erectus*—only values.

Age at M1 eruption is available only for the early African *H. erectus* remains. Basal metabolic rate (BMR) is calculated by using the Oxford equations for prime adults (18 to 30 years) and the average body weight of each species. The average of male ($16 \times \text{weight} + 545$) and female ($13.1 \times \text{weight} + 558$) equations is used (61). TDEE range is calculated as $\text{TDEE} = \text{BMR} \times \text{PAL}$ (physical activity level). A range of PALs from apelike (1.7) (137) to human-like (1.9; the mean of male, 1.98, and female, 1.82, averages for subsistence populations) (138) are used. Lower mean values for *Pan* have been reported (1.5) (139), but given the high range of variation we use the more conservative values.

	<i>A. afarensis</i> versus non-<i>erectus</i> early <i>Homo</i>	<i>A. afarensis</i> versus early <i>H. erectus</i> (<i>H.e.</i>)	African <i>H. erectus</i> versus non-<i>erectus</i> early <i>Homo</i>
Average brain size [source (10)]	<i>Homo</i> larger <i>H.</i> = 629 <i>A.</i> = 478	<i>H. erectus</i> larger <i>H.e.</i> = 787/863 <i>A.</i> = 478	<i>H. erectus</i> larger <i>H.e.</i> = 863 <i>H.</i> = 629
Average body mass [sources (24, 60)]	<i>Homo</i> larger <i>H.</i> = 44 <i>A.</i> = 40	<i>H. erectus</i> larger <i>H.e.</i> = 52/55 <i>A.</i> = 40	<i>H. erectus</i> larger <i>H.e.</i> = 55 <i>H.</i> = 44
TDEE	<i>Homo</i> larger <i>H.</i> = 2026–2264 <i>A.</i> = 1927–2153	<i>H. erectus</i> larger <i>H.e.</i> = 2224–2568 <i>A.</i> = 1927–2153	<i>H. erectus</i> larger <i>H.e.</i> = 2298–2568 <i>H.</i> = 2026–2264
Strength proportions humero-femoral [sources (133, 134)]	Both similar to <i>Pan</i>	Relatively less strong humerus in <i>H. erectus</i>	Relatively less strong humerus in <i>H. erectus</i>
Limb proportions humero-femoral length hind limb to body mass [sources (24, 60)]	Same Same	Same Same	Same Same
Sexual dimorphism, brains [sex designations as per source (10)]	<i>Homo</i> less dimorphic <i>H.</i> = 1.05 (σ 625; η 590) <i>A.</i> = 1.3 (σ 507; η 400)	<i>H. erectus</i> less dimorphic <i>H.e.</i> = 1.15/1.2 (σ 840/924; η 730/770) <i>A.</i> = 1.3 (σ 507; η 400)	<i>H. erectus</i> more dimorphic <i>H.e.</i> = 1.2 (σ 924; η 770) <i>H.</i> = 1.05 (σ 625; η 590)
Sexual dimorphism, bodies [using associated skels; source (10)]	<i>Homo</i> more dimorphic <i>H.</i> = 1.39 (σ 46; η 33) <i>A.</i> = 1.32 (σ 39; η 29.5)	<i>H. erectus</i> less dimorphic <i>H.e.</i> = 1.06/1.0 (σ 50/51; η 47/51) <i>A.</i> = 1.32 (σ 39; η 29.5)	<i>H. erectus</i> less dimorphic <i>H.e.</i> = 1.0 (σ 51; η 51) <i>H.</i> = 1.39 (σ 46; η 33)
Sexual dimorphism, bodies [sex designations source (24)]	<i>Homo</i> more dimorphic <i>H.</i> = 1.77 (σ 56.7; η 31.9) <i>A.</i> = 1.32 (σ 39; η 29.5)	<i>H. erectus</i> less dimorphic <i>H.e.</i> = 1.20/1.25 (σ 55.8/60.4; η 46.2/48.2) <i>A.</i> = 1.32 (σ 39; η 29.5)	<i>H. erectus</i> less dimorphic <i>H.e.</i> = 1.25 (σ 60.4; η 48.2) <i>H.</i> = 1.77 (σ 56.7; η 31.9)
Dental microwear [source (86)]	Unremarkable M surface complexity	Unremarkable M surface complexity with substantial variation and more small features	Unremarkable M surface complexity with substantial variation and more small features
Dental isotope data $\delta^{13}\text{C}$ [source (21, 22)]	<i>Homo</i> slightly less negative <i>A.</i> = -7.4 <i>H.</i> = -6.98	<i>H. erectus</i> less negative <i>H.e.</i> = -4.17 <i>A.</i> = -7.4	<i>H. erectus</i> less negative <i>H. erectus</i> = -4.17 <i>H.</i> = -6.98
Age at M1 eruption [source (135)]	Unknown <i>H.</i> = unknown <i>A.</i> = 2.9–3.6 years	Later in <i>H. erectus</i> <i>H.e.</i> = 4.4–4.5 years <i>A.</i> = 2.9–3.6 years	Unknown <i>H.e.</i> = 4.4–4.5 years <i>H.</i> = unknown
Ranging and stone transport distances [sources (4, 83)]	Early <i>Homo</i> 10s to 100s of m from 2.6 to 2.3 Ma and further by $\sim$ 1.95 Ma; no evidence of transport in <i>A.</i>	<i>H. erectus</i> and possibly other early <i>Homo</i> transport rock 12 to 13 km after 1.95 Ma; no evidence of transport in <i>A.</i>	<i>H. erectus</i> perhaps transports rock further. <i>H.e.</i> sites from Africa to E. Asia by 1.7 Ma
Cutmarked/percussion marked bone	Episodic 2.6 to 2.0 Ma; a possible occurrence in <i>A. afarensis</i>	Ubiquitous after 2.0 Ma	Similar? Episodic 2.6 to 2.0 Ma; ubiquitous after 2.0 Ma
Tool technologies [sources (94, 136)]	None prior to 2.6 Ma when Oldowan appears	<i>H. erectus</i> associated with Oldowan + Acheulean after 1.76 Ma	Both associated with Oldowan until at least 1.76 Ma

Table 3. Inferences regarding physiological, behavioral, and ecological differences between *Australopithecus* and *Homo*. These comparisons have their basis in empirical evidence noted in Table 2 and comparative biological models noted in the text.

	<i>Australopithecus</i> versus non-erectus early <i>Homo</i>	<i>Australopithecus</i> versus Early <i>H. erectus</i>	<i>H. erectus</i> versus non-erectus early <i>Homo</i>
Energetic requirements (brains and bodies)	<i>H.</i> greater on average	<i>H.e.</i> greater	<i>H.e.</i> greater on average
Developmental rate (teeth) [source (135)]	unknown	<i>H.e.</i> slower than <i>A.</i> but faster than <i>H. sapiens</i>	unknown
Developmental rate (bodies) [sources (46, 97)]	unknown	<i>H.e.</i> body relatively faster than teeth; intermediate btwn <i>Pan</i> and <i>H. sapiens</i>	unknown
Nutritional environment/diet (from teeth) [source (86)]	Tougher, less brittle food items in <i>H.</i> Possibly more incisal preparation in <i>H.</i>	Tougher, less brittle food items in <i>H.e.</i> Greater diet breadth in <i>H.e.</i> than <i>A.</i>	Tougher, less brittle food items in <i>H.e.</i> Greater diet breadth in <i>H.e.</i>
Nutritional environment/diet (from brains/bodies)	<i>H.</i> somewhat higher quality	<i>H.e.</i> higher quality	<i>H.e.</i> probably higher quality
Nutritional environment/diet (from archaeology)	<i>H.</i> greater use of animal products?	<i>H.e.</i> more significant use of animal products	<i>H.e.</i> likely greater use of animal products
Nutritional environment/diet (from isotopes) [sources (21, 22)]	<i>A. afar.</i> mixed C ₃ /C ₄ feeder; broad range	Substantially wider C ₃ /C ₄ diet in <i>A. afar.</i> than in <i>H.e.</i>	Similar $\delta^{13}\text{C}$ dietary breadth in <i>H.e.</i> and other early <i>Homo</i> , but little overlap: more C ₄ resources in <i>H.e.</i>
Locomotor repertoire	Both with significant arboreal component	<i>H.e.</i> strongly terrestrial	<i>H.e.</i> more terrestrial
Home range (based on body size, site spatial distribution, and stone transport)	? – somewhat larger in <i>H.</i> due to larger body size, but similar based on site distribution	<i>H.e.</i> larger according to all three variables	<i>H.e.</i> larger according to all three variables
Extrinsic mortality	? – possibly lower in <i>H.</i> given larger body size	Lower in <i>H.e.</i>	Possibly lower in early African <i>H.e.</i> but not for the Dmanisi sample
Developmental plasticity	unknown	Greater in <i>H.e.</i>	Greater in <i>H.e.</i>
Body composition	Larger brains in <i>H.</i> but similar adiposity	Larger brains in <i>H.e.</i> and greater adiposity	Larger brains in <i>H.e.</i> and possibly greater adiposity
Cooperative breeding (alloparenting) [source (104)]	? – possibly more cooperative breeding in <i>H.</i>	<i>H.e.</i> more cooperative breeding necessitated by larger average brain size	<i>H.e.</i> more cooperative breeding necessitated by larger average brain size
Cooperative foraging and carcass acquisition/hunting	? – possibly greater in <i>H.</i>	Likely greater cooperation based on diet shift in <i>H.e.</i>	Likely greater cooperation based on diet shift in <i>H.e.</i>

and C₄ grass-dominated open vegetation habitats (1, 63–65). Accordingly, the spread of African savanna grasslands set the selection pressures that favored stone toolmaking, increased carnivory, and other adaptive characteristics of early *Homo* as a member of the African arid-adapted fauna (64–66). However, a current synthesis of stratigraphic, eolian dust, lake, faunal, stable isotopic, volcanologic, and tectonic data results in a far more dynamic picture of East African environments in which fluctuating moisture and aridity, shifting resource regimes, and spatial heterogeneity were the dominant features of the settings in which early *Homo* evolved (4, 67–70). In contrast to the traditional model of a stable or progressively arid savanna, evidence of climate and landscape variability highlights a different set of adaptive problems in which capacities to buffer and adjust to environmental dynamics at diverse temporal and spatial scales were at a

premium in hominin and other contemporaneous animal populations (67, 71).

Although eolian dust in marine drill cores and limited isotopic data sets from the Turkana Basin, Kenya, have previously been used to emphasize the aridity trend between 3.0 and 1.5 Ma (64), the broader range of data now available emphasizes the wide diversity of vegetational settings and moisture regimes in which *Homo* emerged (72–76) (Fig. 1, D and E). Improved efforts to quantify past and present East African habitats consider savanna to comprise from 5 to 80% woody cover (2), illustrating the potential role of highly diverse habitats in creating speciation opportunities and selective conditions favoring hominin adaptive versatility (4, 73, 77, 78).

Large lake fluctuations during times of strengthened monsoon intensity (68, 77, 79, 80) along with volcanism and tectonic impacts (81) were sources of instability in East African landscapes

and ecological settings. The tempo of wet-dry variability for tropical Africa was governed by ~20,000-year cycles of orbital precession, whereas variation in Earth's eccentricity on cycles ~100,000 and 413,000 years long altered the long-term pattern of seasonal precipitation intensity and duration. There is thus a causal connection between seasonal variability and longer phases of climate variability over evolutionary time. The modulation of precession by eccentricity defines a predictive pattern of episodic increases and decreases in seasonal monsoon intensity and alternating periods of high climate variability and shorter periods of relative stability over the past several million years in East Africa (67, 69, 72) (Fig. 1E). Arabian Sea eolian dust flux and Mediterranean sapropel intensity are two high-resolution data sets that exhibit this pattern (69, 72), and stratigraphic sequences at well-studied, early to mid-Pleistocene *Homo* sites at

Box 2. Sympatry and niche partitioning in early *Homo*

The number of contemporaneous species of early *Homo* has proved controversial because of differing approaches to species definition and assumptions concerning niche breadth. A single-species or linear hypothesis of hominin phylogeny prevailed through much of the 20th century, underpinned by the idea that a cultural, toolmaking niche was so broad as to competitively exclude multiple species within *Homo* (140–142). Recent interpretation of the fossil crania from Dmanisi, Georgia, as evidence of a single evolving lineage incorporating all early *Homo*, including *H. erectus* (8), is consistent with this assumption. Following their anatomical analyses, the authors suggested that a tool-mediated widening of the dietary niche in early *Homo* may have impeded niche differentiation.

However, carbon isotopic values for teeth of broadly sympatric representatives of early *Homo* in the Turkana Basin, Kenya (~1.99 to 1.46 Ma), call into question the idea that tool use precludes niche differentiation. These carbon isotopic values range from –2.6 to –9.9 per mil (‰) for all early *Homo*, indicating sufficient resource space to sustain dietary differentiation. This range exceeds the isotopic separation of 3.5 to 4.3‰ seen in sympatric lineages of fossil murine rodents in the late Miocene Siwalik sequence of Pakistan (143) and the 1.3‰ separation in the means for hair samples of sympatric West African chimpanzees and gorillas (combined range for the two species is 3.7‰) (144). Furthermore, an isotopic difference of 2.8‰ between the means for *H. erectus* ($N = 10$; = –4.3‰) and non-*erectus Homo* ($N = 15$; = –7.1‰) in the Turkana Basin is nearly identical to the difference of 2.9‰ between the means of Turkana Basin *H. erectus* and *Paranthropus boisei*: $N = 27$; = –1.4‰) (18), which suggests the potential for niche partitioning within early *Homo*, all of whom may have been toolmakers.

Additionally, ecological and genetic studies in other organisms provide models for the coexistence of closely related taxa with similar diets and have potential implications for taxonomic diversity in early *Homo*. For example, widespread and highly mobile populations of large-bodied East African giraffe are now considered to compose at least two and possibly five to eight distinct species (145–147). They exhibit relatively deep genetic differentiation in mitochondrial and nuclear DNA consistent with trait differences (e.g., ossicone number) and reproductive isolation in the absence of obvious geographic barriers (148). Maintenance of sympatric taxonomic diversity in the face of overlapping diets is also evident in diverse ungulates and carnivores [e.g., (149, 150)].

These observations, taken together with evidence that *H. erectus* continues in both Africa and Asia after 1.4 Ma yet does not include the morphologies of either the 1470 or 1813 group and that the 1813 group lasts to at least 1.4 Ma in Africa (32), support the view that taxonomic diversity sustained by ecological differentiation did characterize *Homo* between ~2.0 and 1.4 Ma. We thus conclude that tool-assisted dietary flexibility in early *Homo* need not have led to competitive exclusion of multiple species of *Homo*. Such flexibility could instead have favored opportunities for niche differentiation through, for example, seasonal resource partitioning, population size variations in multiple taxa in response to different climate regimes, and differential use of the habitats [including mesic refugia (151) and dry grasslands (152)] that are evident during the time of East African early *Homo*.

East Turkana, Olduvai, and Olorgesailie indicate that this high/low-variability pattern was imprinted on the geological landscapes of East Africa (67, 82–85).

Although certain data sets [e.g., eolian dust and carbon isotope ratio ($\delta^{13}\text{C}$) of soil carbonates] are keenly sensitive to aridity and others (e.g., distribution of lacustrine deposits and diatom stratigraphies) to moisture and lake expansion, the shifting pattern of environmental dynamics over seasonal-to-orbital time scales becomes apparent when uniting the multiple indicators (67). The alternation of high and low climate variability implies that periods of relatively stable environment and the aridity trend were interrupted by lengthy intervals of pronounced habitat unpredictability and resource uncertainty. Although individual organisms experienced extensive seasonal fluctuation, persistent gene pools evolved in the context of long-term re-vamping of resource landscapes and inconsistent timing and intensity of seasonal rainfall in tropical Africa. Accordingly, environmental instability, which included heightened aridity and humidity phases, defined the overall adaptive setting in which key benchmarks of dietary, developmental, cognitive, and social adaptability evolved in early *Homo*.

The paleobiology of early *Homo*

We argue that the origin and evolution of early *Homo* is related to the accommodation of these novel and/or unpredictable environments over time and space. Specifically, increases in average body and brain size and changing dental size coupled with increased toolmaking and stone transport suggest dietary expansion, developmental plasticity, cognitive evolution, and social investments

(see Tables 1 to 3 for relevant data). Together these features and behaviors enabled successful accommodation of these changing environments.

Diet, stone transport, and toolmaking

Isotopic analysis indicates a shift from reliance on C_3 -based foods in early *Australopithecus* (~4 Ma) to a more diverse diet incorporating a broader range of C_3 - and C_4 -based foods in both *Australopithecus* and *Homo* lineages but in different proportions (21, 22) (Fig. 1C). In the same geographical area, East African *Homo* has a diet that is 78% broader than contemporaneous *Paranthropus*, which specialized in more C_4 [and/or crassulacean acid metabolism (CAM)] foods. However, East African *Homo* has a dietary breadth that is only 63% that of *A. afarensis* and 79% that of *A. africanus*. This suggests that it is not dietary breadth as reflected in isotopic breadth that was important in the evolution of *Homo*, but rather the inclusion of a broader range of food stuffs within a narrower isotopic range. Hard evidence for this in early *Homo* dental morphology suggests a shift toward incisal preparation and molar shearing, which may indicate the incorporation of tough-plant products or animal tissues (86). In *H. erectus*, smaller incisors and molars, together with a broader range of microwear textural complexity and a smaller average feature size (Table 1), implies a more diverse diet, including the incorporation of increased meat consumption and/or other tough foods as well as tool use in food preparation.

The archaeological record is consistent with this interpretation. Although tool cut marks have been found on large animal bones by 2.58 Ma (87) and possibly earlier (88), evidence of stone

tool-assisted foraging is intermittent (stratigraphically discontinuous) before 2.0 Ma (2). Core-flake-hammerstone technology (Oldowan) is temporally persistent beginning ~2.0 Ma; along with the acquisition of large animal tissues at least partly by hunting and butchery, the exploitation of diverse terrestrial and aquatic resources, and tool-edge wear consistent with processing underground tubers and roots (89–92). Stone tools were transported from as far away as 12 km from source (93), which underscores the energetic trade-off between the cost of stone transport and the energetic returns from tool use. The Oldowan also provided the technological basis for expansion into southern and northern Africa and western Asia by 1.85 Ma, and the appearance of the Acheulean by 1.76 Ma (94) may have further enhanced adaptive potential.

These fossil, isotopic, and archaeological features suggest substantial differences between early *Homo* and earlier hominins, as well as contemporaneous *Paranthropus*, that imply flexibility in accommodating to habitat and resource diversity and unpredictability in eastern Africa and beyond. This trend, which begins with early *Homo* and intensifies in *H. erectus*, is also consistent with niche partitioning and the existence of contemporaneous hominin taxa in this period (Box 2).

Body size and developmental plasticity

Body-size increase and developmental accommodation as inferred from intrataxon variation in body size also indicate adaptive flexibility. There is an increase in average body size from *Australopithecus* to early *Homo* to *H. erectus* (Table 1), as well as substantial intrataxon size

variation. The range in body size across paleopopulations, particularly in *H. erectus*, is similar to that found in modern humans, where it is known to be a complex reflection of mortality probability and nutrition (95, 96). In living humans, these variables interact to affect both the duration and the speed of ontogeny, and this developmental flexibility allows a large reaction norm of body sizes. Relatively large size in humans is found in environments of high nutritional sufficiency, and selection for later maturation and extended longevity occur only in situations of relatively low extrinsic mortality risk (e.g., low predation risk or parasite load). *H. erectus* ontogeny was likely somewhat slower than in *Australopithecus* or non-*erectus* early *Homo* but considerably faster than in modern humans (97, 98). This suggests that *H. erectus* may have been able to reduce mortality risk in relation to other hominins through social or other factors.

Larger body size in *Homo* in relation to *Australopithecus* undoubtedly reflects nutritional sufficiency resulting from tool use, social cooperation, and a higher-quality diet. This interspecific size increase does not preclude the effect of habitat variation among populations of, for example, *H. erectus*, where low-quality habitats would be associated with smaller body size, as is the case in other primates (99–101). Once established, larger body size provides a greater range of phenotypic adaptive flexibility in response to environmental circumstances. Across mammals, larger body size also equates with larger home range sizes, which would have been exaggerated further if the *Homo* diet was at the more carnivorous end of the omnivorous spectrum (43, 102). Large home ranges imply increased total daily energy expenditure (TDEE) in relation to body size and a greater

reproductive investment (greater lifetime reproductive output) (24, 103). TDEE may have been even higher in *H. erectus* because of larger brain size in this species, implying efficiency in obtaining a high-quality calorie-rich diet.

Encephalization, cognitive evolution, and social investment

Average brain size also increases from *Australopithecus* to early *Homo* to *H. erectus*. Large brains require an increase in total energy and/or a reduction in energy allocation to other expensive functions, such as maintenance (smaller guts), locomotion (efficient bipedal locomotion), or production (slower growth and reproduction) (104–110). All of these factors are observed or inferred in the evolution of *Homo*.

There is also some indication that cooperation in the form of allocare is directly related to increased brain size. For example, social carnivores show a tendency in this direction, where a modest amount of cooperation is correlated with larger brain sizes in social carnivores compared with their more isolationist congeners (111). New work also suggests that, because of the extended ontogenetic periods necessary for the growth of larger-bodied and -brained offspring, the great apes are at the demographic limit for brain size increase. Larger-brained hominins (over about 700 cm³ in average size) could not reproduce fast enough to sustain population numbers without greater cooperative care that would provide extra resources to the mother and result in earlier weaning, shorter interbirth intervals, and higher overall fertility (105). Other factors, such as adiposity, may also have been important to brain growth early in ontogeny (112). Although we are currently limited in our ability to gauge soft-tissue fea-

tures from the fossil record, adiposity and sex differences in relative adiposity are critical components of human abilities to disperse into myriad environments.

Thus, the increase in average body and brain size from *Australopithecus* to early *Homo* to *H. erectus* is consistent with a greater control over or amelioration of mortality risk and increased nutritional sufficiency. Increasing cultural mediation and enhanced niche construction (4, 71, 113, 114), through technology and social factors such as food sharing or allocare, would be essential to buffer against fluctuating climatic conditions, reduce predation pressure and extrinsic mortality risk, and insure greater food availability.

Niche construction and dispersal

These lines of reasoning suggest that there were a variety of strategies available to early *Homo* that enabled adaptive flexibility in the context of climatic variability and dispersal to new habitats. We emphasize that flexibility is just one possible reaction to these environmental challenges and need not be the one that all or even most animals took at this time. Lessons from extant comparators suggest that intraspecific phenotypic plasticity provides a more rapid response to environmental challenges than genetic change but that genetic change can follow (95). We expect that this is precisely what occurred with the evolution of *Homo*. Different species (1470 group, 1813 group, and *H. erectus*) used different strategies. In the face of a dynamic and fluctuating environment, we suggest that the unique combination of larger brain size, the potential for diverse body sizes, inferred dietary flexibility, and cooperation enabled *H. erectus* to attain a level of niche construction and adaptive versatility that allowed this species to outpace its congeners.

This same adaptive flexibility was likely essential to the expansion of *Homo* out of Africa and into Eurasia. The limited available fossil evidence is consistent with this in suggesting that *H. erectus* was the first hominin to leave Africa, reaching Dmanisi (Georgia) by 1.85 Ma and Sangiran (Java, Indonesia) by 1.66 Ma (Fig. 2) (115, 116). Two incisors from Yuanmou (Yunnan, China; 1.71 Ma) are similar to those of KNM-ER 15000 (Nariokotome, West Turkana, Kenya; 1.6 Ma) and have also been assigned to *H. erectus*, although their hominin affinities are contested (55, 57). Regardless, archaeological sites in the Nihewan basin of northern China (Hebei Province) at 1.66 Ma confirm that hominins in eastern Asia spanned a variety of habitats from 40°N (Nihewan basin) to 7°S (Sangiran, Java, Indonesia) (116).

Given the sparse record, the possibility remains, of course, that *H. erectus* was not the first or only hominin to disperse from Africa. Primitive aspects of the postcranial skeleton of the relatively recent island hominin *Homo floresiensis* raise the possibility of a pre-*erectus* hominin in eastern Asia (117), although more-recent work suggests that *H. floresiensis* was derived from an *H. erectus* ancestor (118–121). The fossil and

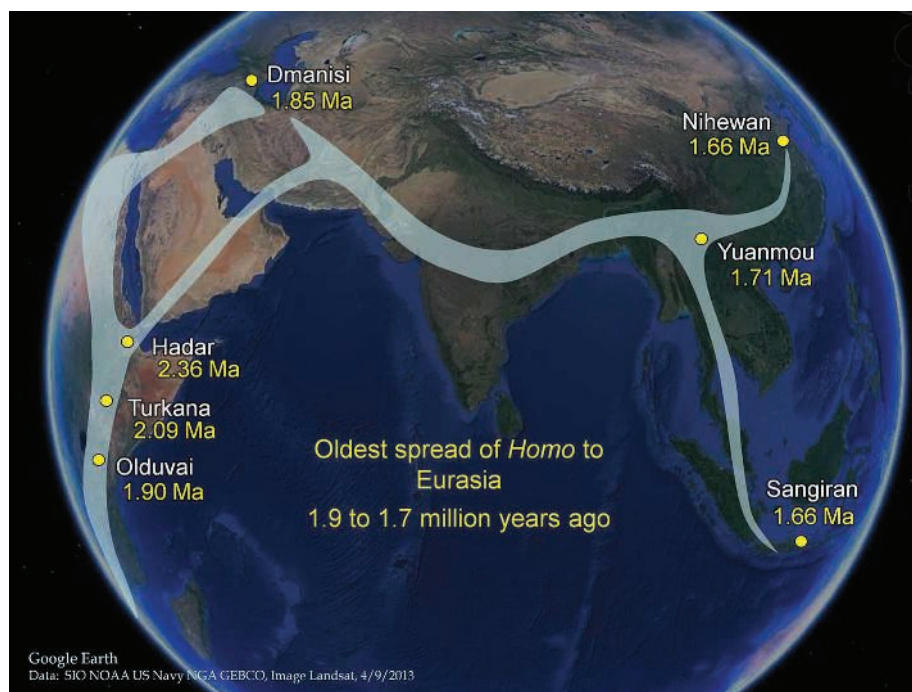


Fig. 2. Key sites and first appearances in the dispersal of early *Homo* from Africa.

archaeological evidence to date as well as inferred features of adaptive flexibility all point to *H. erectus* as the first disperser.

Reduction of mortality risk and increased nutritional sufficiency implied by increasing body and brain sizes, and enhanced niche construction and ranging implied by both the archaeological and fossil records, suggest a level of adaptive flexibility that ultimately allowed the dispersal and range expansion by *H. erectus*. However, we caution that, even if *H. erectus* was the first and only to disperse, that geographic expansion was likely to have been episodic and to have involved multiple populations and back migrations. Figure 2 implies only the ultimate direction of dispersal initially from west to east, not the specific mechanism or complexity of that dispersal.

Conclusion: New frameworks and unresolved questions

A suite of morphological and behavioral traits once considered to define the origin of the genus *Homo* or of earliest *H. erectus* evolved not as an integrated package but over a prolonged time frame that encompassed species of *Australopithecus*, early *Homo*, *H. erectus*, and later *Homo*. The idea of an integrated package of traits in early *Homo* has been thought to anticipate the adaptive characteristics of *H. sapiens* and to include reduced face and teeth, a substantial increase in brain size, body proportions characterized by an elongated hind limb and shortened forelimb, essentially modern hand functional morphology, dependence on toolmaking and culture with incipient language capabilities, dietary expansion, persistent carnivory and systematic hunting, narrow hips with implications for the

birth of altricial young, prolonged life history compared with extant apes, and cooperative food-sharing focused at a home base (15, 122–125). New fossil and archaeological data summarized here allow refined perspectives on the morphological variation and pacing of evolutionary change in the *Homo* clade. These empirical findings, coupled with interpretive models drawn from developmental and comparative biology and behavioral ecology, now require the disentangling of this package of traits (Fig. 3).

An important, continuing goal is to develop a more refined understanding of exactly what adaptive features did originate with early *Homo*. According to present data, facial and dental reduction defines the earliest members of the genus between 2.4 and 2.0 Ma. Cranial capacity expanded by 2.0 Ma. A greater yet varied degree of brain enlargement correlated with body size increase is expressed in early *H. erectus* between 1.9 and 1.5 Ma, although estimates of the degree of encephalization overlap with those of *Australopithecus*. However, brain expansion independent of body size appears to be most strongly expressed later, between 800 and 200 thousand years ago. A relatively elongated hind limb is present in *A. afarensis* (by 3.9 Ma) and in later *Australopithecus* (*A. africanus*, *A. garhi*, and *A. sediba*) but not in *Ardipithecus* (4.4 Ma). Absolutely longer and strongly built femora evolved between 1.9 and 1.5 Ma, coinciding with early *H. erectus*. Stone technology at ~2.6 Ma may predate the origin of *Homo*, whereas cultural capabilities of the early Pleistocene led to highly persistent traditions of toolmaking rather than an innovative, cumulative culture linked to symbolic behavior typical of the latter part of the Pleistocene. Transversely oriented hips and a

broad pelvis persisted until *H. sapiens*, although a brain consistently >700 cm³, which occurred after ~1.8 Ma, connotes altricial neonates and heightened cooperation among *H. erectus* adults. Based on first molar dental histology and eruption, the tempo of life history was slower in *H. erectus* than in *Australopithecus* yet was similar to that of extant great apes. Far more prolonged phasing of growth typical of *H. sapiens*, with implications for intensive social cooperation, is evident in the middle Pleistocene, which is also when definitive evidence of hearths and shelters occurs in the archaeological record, implying strong centrally located social cooperation. The traits associated with the origin of *Homo* and of *H. erectus* thus evidently did not approximate the integrated complex of adaptations found in *H. sapiens*.

The evolution of early *Homo*, moreover, was associated with recurrent periods of intensified moist-dry variability (Fig. 1E). Dynamic environments favored evolutionary experimentation and the coupling and uncoupling of biological variables (71, 126), which governed against any simple transition from *Australopithecus* to *Homo*. We maintain that the East African record to date preserves three distinct taxa of early *Homo*, including *H. erectus*, although the issues that arise from recent discoveries elsewhere at Malapa and Dmanisi hint at the intriguing shuffling of derived and plesiomorphic traits and biological variables that likely characterized the early evolution of *Homo*.

Developmental plasticity and ecological versatility were at a premium in the habitats in which early *Homo* evolved. Although plasticity across biological levels (molecular to behavioral) was favored in dynamic habitats, both extrinsic (e.g., environmental) factors as well as biological and social feedback mechanisms were complexly entwined in the evolution of *Homo* and can no longer stand as alternative explanatory hypotheses (4, 61). Understanding the processes by which adaptability evolved in *Homo* and exactly how various traits contributed to plasticity during the evolution of the genus are important future challenges.

Critical foci for future research on the paleobiology of early *Homo* are numerous. To cite four examples, first, the field is always well served by new fossil and archaeological finds. Larger fossil samples between 2.5 and 1.5 Ma will be necessary to assess the taxonomic diversity of early *Homo* and to determine the temporal and spatial integrity of the morphological groups. Second, comparative mammalian studies focused on population structure, genetic isolation, niche differentiation, and the variables enabling the coexistence of congeneric taxa will help build more effective models for understanding morphological groups and diversity in early *Homo*. Third, much remains to be learned about encephalization in early *Homo*, the degree of plasticity in body and brain size, and how these variables were related to paleoenvironmental variables (e.g., shifting resource abundance). Last, interpretations concerning early *Homo* rely on the comparative

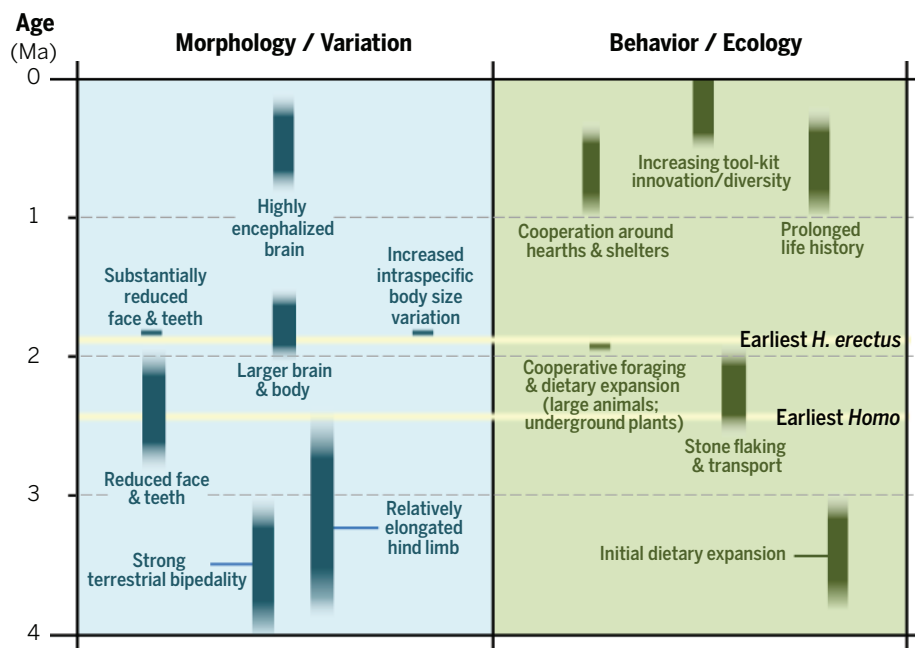


Fig. 3. Evolutionary timeline of important anatomical, behavioral, and life history characteristics that were once thought to be associated with the origin of the genus *Homo* or earliest *H. erectus*.

biology of a wide range of mammals (including humans) in order to test and develop robust models of the intricate relationships between energetics, life history, brain and body size, diet, mortality, and resource variability across temporal and spatial scales. A refined understanding of these relationships will enable the union of many disciplines to yield a deeper understanding of human evolution.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/344/6192/1236828/suppl/DC1
Supplementary Text
Tables S1 to S3

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RESEARCH ARTICLES

EXOPLANET DETECTION

A terrestrial planet in a ~1-AU orbit around one member of a ~15-AU binary

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Using gravitational microlensing, we detected a cold terrestrial planet orbiting one member of a binary star system. The planet has low mass (twice Earth's) and lies projected at ~0.8 astronomical units (AU) from its host star, about the distance between Earth and the Sun. However, the planet's temperature is much lower, <60 Kelvin, because the host star is only 0.10 to 0.15 solar masses and therefore more than 400 times less luminous than the Sun. The host itself orbits a slightly more massive companion with projected separation of 10 to 15 AU. This detection is consistent with such systems being very common. Straightforward modification of current microlensing search strategies could increase sensitivity to planets in binary systems. With more detections, such binary-star planetary systems could constrain models of planet formation and evolution.

Although at least half of all stars are in binary or multiple systems, the overwhelming majority of detected exoplanets orbit single stars—or at least stars whose companions have not been detected or are so far from the planet's host as to be physically irrelevant. Because binary stars are so common, theories of planet formation and orbital evolution should be strongly constrained by the observed frequency and parameter distributions of planets in these systems. For example, the presence of a relatively near companion might truncate or disrupt the protoplanetary disk that is thought to be the planet's birthplace. Exploring planets in binary systems is therefore an important frontier.

Microlensing is complementary to other planet-finding techniques in terms of sensitivity as functions of planet-host separation, host mass, planet mass, and planet-host position within our Galaxy. The basic scale of microlensing phenomena is set by the Einstein radius (R)

$$\theta_E \equiv \sqrt{\kappa M \pi_{\text{rel}}} \quad \kappa \equiv \frac{4G}{c^2 \text{AU}} \simeq 8.1 \frac{\text{mas}}{M_\odot} \quad (1)$$

where M is the lens mass, $\pi_{\text{rel}} = \text{AU}(D_L^{-1} - D_S^{-1})$ is the lens-source relative parallax, D_L and D_S are the distances to the lens and the source, respectively, mas is 10^{-3} arcseconds, and $M_\odot$ is the mass of the Sun. If two stars are perfectly aligned on the sky, then the gravity of the one

in front ("lens") bends the light from the one in back ("source") into an annulus ("Einstein ring") of radius θ_E and width twice θ_E , where θ_E is the source angular radius. If the lens-source separation $\Delta\theta_{LS}$ is nonzero but still $\Delta\theta_{LS} \ll \theta_E$, then the source light is broken up into two images, one inside and the other outside the Einstein ring. The two images are separated by $2\theta_E \sim O(\text{mas})$ and so are not resolvable with current telescopes. However, the combined area of the images is larger than the source and so appears brighter by the magnification A , which scales very nearly as $(\Delta\theta_{LS}/\theta_E)^{-1} \equiv u^{-1}$ for $u \ll 0.5$. Hence, as the lens passes by the projected position of the source, the magnification increases and then decreases, creating a "microlensing event." Currently, more than 2000 such events are discovered each year. If the lens has a planet, and one of the two images passes near this planet, its gravity further deflects the light, changing the light curve and thereby betraying its presence. Planet sensitivity peaks over the range $0.6\theta_E \ll a_1/D_L \ll 1.6\theta_E$, which corresponds to a planet-host physical separation a_1

$$a_1 \sim r_E \equiv D_L \theta_E \sim 3.5 \text{ AU} \sqrt{\frac{M}{M_\odot}} \quad (2)$$

for typical event parameters. Because this is a two-dimensional (2D) projection of a 3D ellipti-

cal orbit, with semimajor axis a , the semimajor axis is typically larger by $a/a_1 \sim \sqrt{3/2}$, i.e., $a \sim 4.3 \text{ AU}$ for a solar-mass host. By contrast, the "snow line," outside of which ices can condense and so promote the growth of giant planets, is 2.7 AU in the solar system and is generally believed to increase monotonically with host mass [e.g., (2)]. Hence, microlensing probes planets in the cold outer regions, far from their host stars. By contrast, the radial velocity (RV) and transit techniques are most sensitive to planets much closer to hosts, and imaging is sensitive to planets much farther out. Because microlensing does not depend on host (or planet) light, it is sensitive to low-luminosity (even nonluminous) hosts and to systems that are many kiloparsecs (kpc) away. Finally, in sharp contrast to all other methods, the amplitude of the microlensing signal does not necessarily decline as the planet mass decreases. This does not mean that microlensing is equally sensitive to all planet masses: The linear extent of the planetary caustic (closed curve of formally infinite magnification) declines as $\sqrt{q}$, where $q = m_p/M$, which reduces both

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the probability and duration of perturbation by $\sqrt{q}$. However, when these perturbations occur, they can be robustly detected [see the review by Gaudi (3)].

Discovery and characterization of planet in binary system

Microensing event OGLE-2013-BLG-0341 was detected by the Optical Gravitational Lensing Exper-

Table 1. Observatories. CTIO-SMARTS (Cerro Tololo Inter-American Observatory, Small and Moderate Aperture Research Telescope System); PEST (Perth Extrasolar Survey Telescope); I band (centered near 810 nm); R band (centered near 670 nm); RI band (combined R and I bands); N (no filter used).

Name	Location	Diameter (m)	Filter
OGLE	Chile	1.3	I
MOA	New Zealand	1.8	RI
Wise	Israel	1.0	I
Auckland	New Zealand	0.40	R
CTIO-SMARTS	Chile	1.3	I
Farm Cove	New Zealand	0.36	N
PEST	West Australia	0.30	N
Possum	New Zealand	0.36	N
Turitea	New Zealand	0.36	R

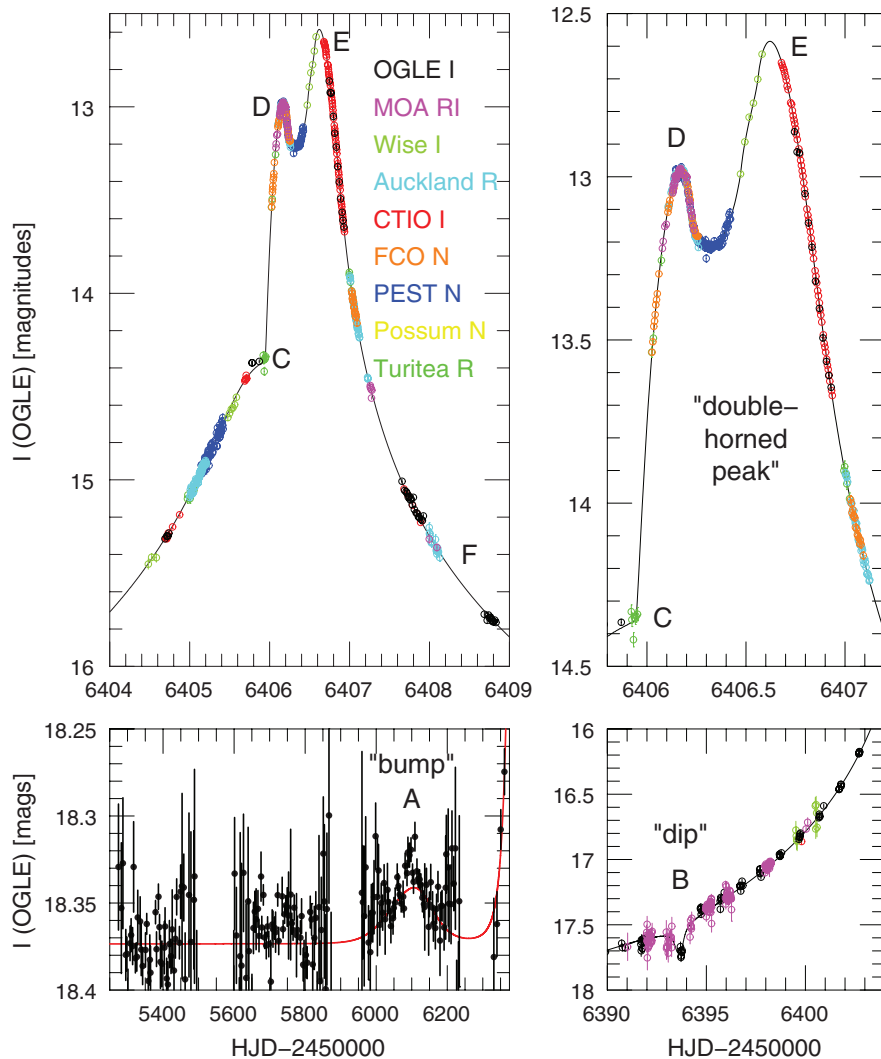


Fig. 1. OGLE-2013-BLG-0341 light curve. (Top) Light-curve features (C to F), induced by main caustic due to binary, are seen as source passes close to planet host. The entrance has a sharp break (C) indicating a caustic crossing, whereas the exit does not (E and F), indicating a cusp exit. (Bottom left) Low-amplitude “bump” (A) due to source’s passage relatively far from binary companion to host, ~300 days earlier. (Bottom right) “Dip” (B) due to planet “annihilating” one of the two main images of the source.

iment (OGLE, Chile) (<http://ogle.astrouw.edu.pl/>) (4) and was also observed in survey mode by two other surveys, Microlensing Observations in Astrophysics (MOA, New Zealand) (www.phys.canterbury.ac.nz/moa/) (5, 6) and the Wise Observatory (Israel) (<http://wise-obs.tau.ac.il/~wingspan/>) (7). It was intensively observed in follow-up mode by six Microlensing Follow Up Network (μ FUN) (<http://www.astronomy.ohio-state.edu/~microfun/>) observatories. See Table 1.

The precise extraction of all parameters requires computationally intensive modeling [see the supplementary materials (8)], but the characteristics relevant to the main scientific implications of this discovery can mostly be derived from inspection of the light curve (Fig. 1). There are three main features, a double-horned peak lasting ~1 day centered at $t \sim 6406.5$ day, an extended, very shallow “bump” at $t \sim 6100$ day, and a very short “dip” at $t \sim 6394$ day. The first two are due to the binary, and the last is due to the planet. Such features have been seen and analyzed previously in many planetary and binary microlensing events; the difference in this case is that (i) they appear together and (ii) there is a subtle interplay between them. The duration of the principal peak is ~65 times shorter than the Einstein diameter crossing time $2 t_E$, where $t_E \equiv \theta_E/\mu_{\text{rel}} \sim 33$ days and μ_{rel} is the lens-source relative angular speed. This peak is therefore due to a very small central caustic, which could in principle be due either to a planet near the Einstein ring or to a companion star that is far from it. The sharp beginning ($t \sim 6406.0$ day) and smooth end ($t > 6407$ day) imply fold-caustic entrance and cusp exit (Fig. 2). This morphology is consistent only with a binary lens. Although the binary companion could be, in theory, either very far inside (“close”) or very far outside (“wide”) the Einstein ring, the early bump at $t \sim 6100$ day confirms the latter interpretation: This bump was generated by the source passing moderately close to the companion a year earlier (Fig. 2). Finally, the small dip at $t \sim 6394$ day can only be caused by a planet that is inside the Einstein ring. Recall that the principal lens creates two images, which are at extrema of the time-delay surface (Fermat’s principle). The outside image is at a minimum of this surface, and the inside image is at a saddle point. A planet sitting at exactly this saddle point will effectively annihilate the image, causing a dip. To generate only a dip and no neighboring bumps, the source must have “threaded” the planetary-caustic structure as it headed toward the central caustic (Fig. 2). The half-crossing time of the dip is $t_{\text{dip}} \sim 0.25$ day. Because the planetary caustic size scales as $(t_{\text{dip}}/t_E) \sim q^{1/2}$, the planet is $q \sim 0.6 \times 10^{-4}$ times less massive than its host. From the fact that the interval between the planetary and binary caustics is $\sim 0.4 t_E$, we calculate that the planet-host separation (normalized to the Einstein radius) is $s_2 \sim 1 - 0.4/2 = 0.8$ from the center of magnification of the system (which in this binary system is very close to the host).

The next step is to transform the dimensionless separations into angles by measuring θ_E , using the source size θ_* as a “ruler” (9). From its

Fig. 2. Geometry of OGLE-2013-BLG-0341 “wide minus” solution. (Bottom) Locations of the host (M_*), planet (M_p) and companion (M_c), and of the caustics (closed curves of formally infinite magnification) that induce strong perturbations in the light curve. Source position is shown at six key times (A to F) corresponding to light-curve features in Fig. 1. **(Middle)** Zoom of planetary caustic (left) and central caustic (right), giving rise to “dip” and main peak seen in Fig. 1. Central caustic and lens positions are shown at two different epochs (“A” and “E”) separated by ~ 300 days, during which it changed its shape and orientation due to binary orbital motion as described in the supplementary materials. **(Top)** Further zoom showing source (yellow) to scale. Blue and red caustics and circles indicate lens geometries at times of “bump” (A) and main peak (D), respectively. One unit on the x axis corresponds to $t_E = 33$ days in time.

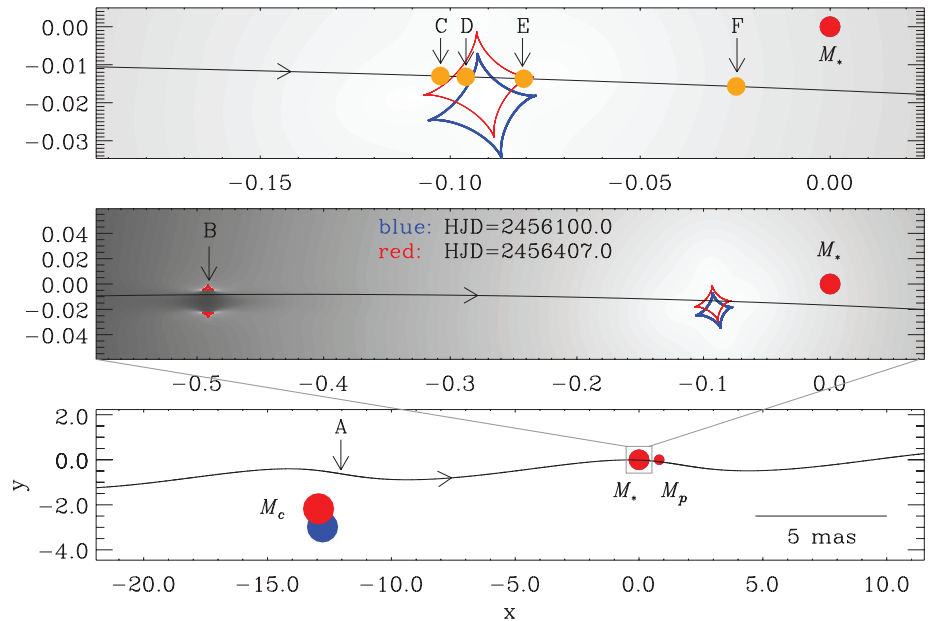
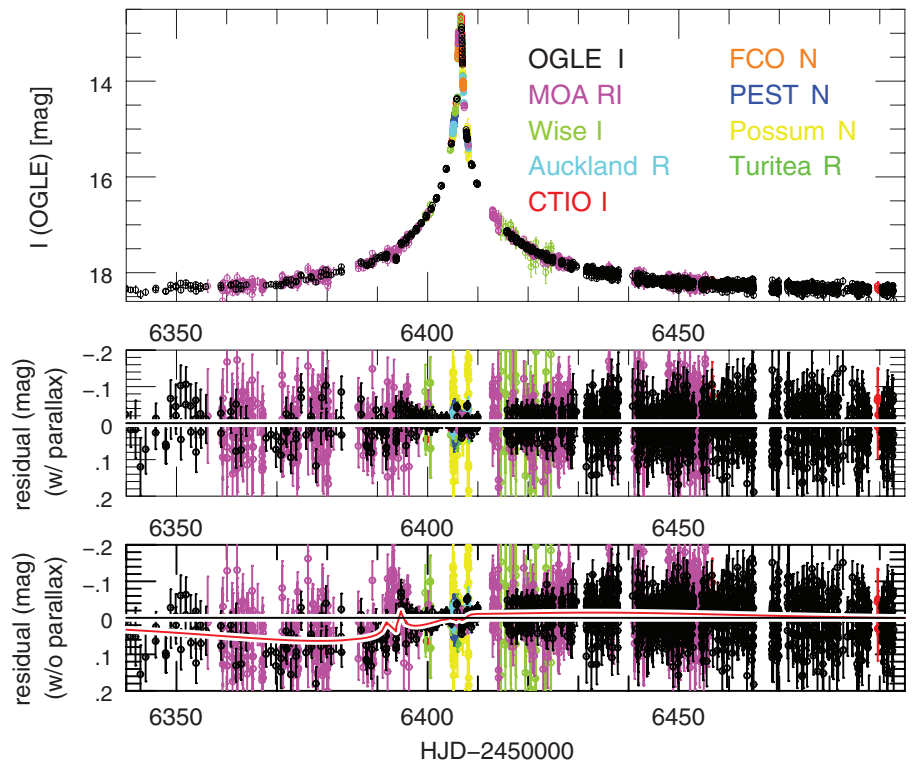


Fig. 3. Full OGLE-2013-BLG-0341 light curve (top) and residuals from “wide minus” model with (middle) and without (bottom), including the parallax effect. Parallax is strongly detected, $\Delta\chi^2 = 730$. Silhouetted black and red curves indicate zero and difference between parallax and no-parallax models, respectively. In contrast to all other crucial light-curve parameters, the parallax effect is not directly visible in the light curve, but only in the residuals. However, as explained in the supplementary materials, an experienced modeler can “read off” from these residuals that $\pi_E \gg 0.7$.



measured color (and thus, because stars are approximate black bodies, surface brightness, S) and flux F , we determine that $\theta_* = \sqrt{F/\pi S} = 2.9 \mu\text{as}$ (8). Comparing the caustic rise time (6405.97 to 6406.17) to the standard mathematical form [steep, then rounded rise totaling 1.7 source-radius crossing times, (10)], and for simplicity ignoring that this entrance is at an angle, we can estimate a source crossing time of $t_* \sim 0.12$ days. The resulting Einstein radius is $\theta_E = (t_E/t_*)\theta_* \sim 0.8$ mas.

The final step is to measure the distance using the “microlens parallax,” $\pi_E \equiv \pi_{\text{rel}}/\theta_E$. This quantifies the amplitude of lens-source relative motion due to reflex motion of Earth’s orbit (scaled to the Einstein radius) and therefore the amplitude of the light-curve deviations due to this effect [see (11), figure 1, for a didactic explanation]. The impact of this effect on the light curve is easily seen (if not quantified) in the residuals to models with and without parallax (see Fig. 3). There is a well-known degeneracy in parallax so-

lutions (labeled “+/-”), depending on which side of the projected position of Earth the lens passes relative to the source (12), and we show (8) that this degeneracy cannot be broken in this case. (We also include in the model orbital motion of the binary about its center of mass. These effects are discussed in detail in the supplementary materials but are too subtle to detect by eye from the light curve.) Thus, we find $\pi_E = 1.0$ or $\pi_E = 0.8$. Then from the definitions of θ_E and π_E , we obtain $\pi_{\text{rel}} = \theta_E \pi_E$ and $M = \theta_E/\kappa \pi_E$ and have calculated

Table 2. Physical parameters of the binary + planet models. Masses, distances, and projected separations for binary + planet system in the two models that are consistent with the microlensing data.

Parameter	Unit	Wide(+)	Wide(−)
M_{total}	$M_{\odot}$	0.2336 0.0182	0.3207 0.0284
M_{host}	$M_{\odot}$	0.1127 0.0089	0.1452 0.0135
M_{planet}	$M_{\oplus}$	1.66 0.18	2.32 0.27
$M_{\text{companion}}$	$M_{\odot}$	0.1209 0.0094	0.1755 0.0155
Distance	kpc	0.911 0.070	1.161 0.093
$a_{\perp, \text{planet-host}}$	AU	0.702 0.022	0.883 0.043
$a_{\perp, \text{companion-host}}$	AU	10.536 0.318	14.013 0.617

the relevant physical parameters that are derived from each of the two solutions (Table 2).

In either case, the planet has mass $m_p \sim 2$ Earth masses ($M_{\oplus}$), and the host is a late M dwarf, with another, slightly more massive, M dwarf as a companion lying at a projected separation of 10 or 14 AU. The entire system lies ~ 1 kpc from the Sun. Simulations of microlensing with realistic planetary systems that include eccentricity and inclination (13) confirm the naive expectation that these projected separations are good proxies for the semimajor axis (after upward adjustment by $\sqrt{3/2}$ to correct for projection effects). Indeed, the relation between a and $a_{\perp}$ is very similar to the relation between m and $m \sin i$ for RV detections. Hence, as with RV masses, this proxy can fail badly in rare individual cases.

Implications for planets in binary star systems

What are the implications? First, while we cannot reliably estimate the frequency of such systems, we can ask the simpler question: If all stars were in such binary/terrestrial-planet systems, how many should have been detected? The detection required (i) a transit of the source by both the planetary ($p \sim 6 \times 10^{-3}$) and central caustics ($p \sim 7 \times 10^{-2}$) and (ii) relatively high-cadence data on a relatively bright star $I_S < 18.5$ to ensure sufficient signal-to-noise ratio to detect the dip. Therefore, if all $I < 18.5$ stars that undergo microlensing events had such planets, we would detect $\sim 0.04\%$ of them. During the 3 years that OGLE-IV has issued alerts, it detected a total of $\sim 10^3$ of $I_S < 18.5$ events in its high-cadence fields, which implies an expectation of 0.4 such planets. This would be compatible with survey results showing that Earths and super-Earths are the most common type of planet orbiting stars with a wide range of masses (14–18) and with predictions from microlensing based on more massive planets orbiting low-mass stars (19).

Second, this result shows that terrestrial planets can exist relatively far (~ 1 AU) from their hosts even if the latter have relatively nearby

< 20 AU binary companions, thus providing an empirical test of models of terrestrial planet formation in such close binaries [e.g., (20–22)].

Third, when combined with the RV detection of a terrestrial ($m_p \sin i = 1.3 M_{\oplus}$) planet orbiting very close (0.04 AU) to α Centauri B (23), which is a solar-type star, it shows that terrestrial planets can form in binaries with diverse properties in terms of host mass and planet-host separation. Although OGLE-2013-BLG-0341LBb was discovered in a search of $\sim 10^3$ microlensing events and α Centauri Bb resulted from intensive observations of a single system, the expected yield in each case (if all stars had similar planets) was roughly unity.

Planets have been discovered in a variety of binary configurations. For example, about seven transiting circumbinary planets have been discovered in Kepler satellite data (24), and two jovian planets have been found in binary systems using RV (25, 26). Microlensing is also sensitive to planets in very different binary configurations, and both current and future surveys are likely to discover these.

Finally, we discuss an extremely interesting aspect of the modeling of OGLE-2013-BLG-0341 that points to the possibility of much greater sensitivity to systems of this type. When the data near the dip are removed and the remaining light curve is fitted for a binary both with and without a planet, the former solution is preferred by $\Delta\chi^2 = 216$ over the no-planet model. That is, although the planet is lighter than the binary by a factor $q < 10^{-4}$, its presence distorts the caustic enough to be noticed in the very high-density observations of the caustic features. Moreover, this model accurately “predicts” the position of the planet. This means that the planet could have been detected even if the source had not passed over the tiny planetary caustic. This passage accounted for $p \sim 1/170$ in the above probability calculation, implying that by probing the central caustic, sensitivity can be improved by $1/p \sim 170$. However, high-density observations (as in Fig. 1) are not routinely taken for binaries. Indeed, μ FUN organized these only because it recognized from the

form of the planetary caustic that the source was headed toward the central-caustic region and sought to exploit this passage to obtain information about the planet. The resultant dense coverage of the binary caustic was inadvertent. Dense follow-up of “ordinary” binaries may then be the best way to probe for planets in binary systems (27). Because there are a comparable number of high-magnification binary compared to apparently single-star events, the additional observing resources required to carry out such follow-up are relatively modest.

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SUPPLEMENTARY MATERIALS

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Figs. S1 to S3
Table S1
References (28–47)

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MOLECULAR KINETICS

Ras activation by SOS: Allosteric regulation by altered fluctuation dynamics

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Activation of the small guanosine triphosphatase H-Ras by the exchange factor Son of Sevenless (SOS) is an important hub for signal transduction. Multiple layers of regulation, through protein and membrane interactions, govern activity of SOS. We characterized the specific activity of individual SOS molecules catalyzing nucleotide exchange in H-Ras. Single-molecule kinetic traces revealed that SOS samples a broad distribution of turnover rates through stochastic fluctuations between distinct, long-lived (more than 100 seconds), functional states. The expected allosteric activation of SOS by Ras–guanosine triphosphate (GTP) was conspicuously absent in the mean rate. However, fluctuations into highly active states were modulated by Ras-GTP. This reveals a mechanism in which functional output may be determined by the dynamical spectrum of rates sampled by a small number of enzymes, rather than the ensemble average.

Cellular membranes organize signal transduction, serving as platforms for protein interactions as well as direct modulators of enzymatic function (1, 2). The activation of lipid-anchored guanosine triphosphatases (GTPases) of the Ras superfamily by cytosolic guanine nucleotide exchange factors (GEFs) represents a broadly important class of membrane-localized signaling reactions. Control of GEF activity is multilayered and involves membrane recruitment, lateral interactions on the membrane surface, as well as allosteric regulation (3, 4). A recurring feature across several

Ras, Rho, and Arf GTPase subfamily GEFs is the release of autoinhibition mediated by GTPase and membrane binding. The molecular mechanisms underlying these regulatory couplings remain poorly understood, in large part because of the intrinsic experimental challenge of working within a membrane environment. We reconstituted the inner-leaflet signaling geometry of Son of Sevenless (SOS)–catalyzed nucleotide exchange in H-Ras on supported membranes that were partitioned into arrays of two-dimensional corrals by lithographically defined chromium diffusion barriers. With this system, we monitored the real-time catalytic activity of individual SOS molecules in order to observe the functional mechanisms of allosteric activation and autoinhibition on the membrane surface.

SOS is widely distributed in mammalian cells. In vivo, inactive cytosolic SOS is recruited to the plasma membrane in response to ligand binding by receptors on the cell surface. There, it activates membrane-tethered Ras by catalyzing the exchange of Ras-bound guanosine diphosphate (GDP) with guanosine triphosphate (GTP), which triggers the mitogen-activated protein kinase (MAPK) cascade (5). SOS is activated by Ras-GTP binding to an allosteric site, located between the Cdc25 and Ras exchanger motif (REM) domains in the catalytic core termed SOScat (C) (Fig. 1A) (6). This allosteric activation depends sensitively on the nucleotide state of Ras (7) and contributes an important aspect of SOS biology. Functionally, this is thought to enable SOS to operate as an analog-to-digital converter through a Ras-GTP positive-feedback loop operating at the membrane, such as during T cell activation (8, 9). However, the nucleotide specificity of allosteric activation of the catalytic site remains poorly understood. SOS activation is autoinhibited by its N-terminal Histone fold (H), Dbl homology

(DH), and Pleckstrin homology (PH) domains (10). Autoinhibition is thought to occur through steric occlusion of the allosteric site, which can be released by interaction of the PH domain with phosphatidylinositol-4,5-bisphosphate (PIP₂) or other negative lipids on the membrane (11, 12). Several SOS mutations, including R552G in the helical linker, lead to weakened autoinhibition, excessive Ras activation, and Noonan Syndrome developmental disorders (13). (Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. In the mutants, other amino acids were substituted at certain locations; for example, R552G indicates that arginine at position 552 was replaced by glycine.)

Most biological and biochemical studies of SOS activity have relied on bulk assays. Physically distinct aspects of SOS regulation, such as membrane recruitment and allosteric modulation of specific catalytic activity, are intrinsically convolved in such observations (supplementary text S1). Furthermore, any stochastic variation among SOS molecules, such as fluctuations between different activity states, is averaged out in the ensemble result. Because many signaling processes in cells involve small numbers of molecules, the ability to average over large ensembles is not a benefit that live cell-signaling networks necessarily enjoy (14–17). Stochastic variation itself, rather than ensemble average properties, can be a viable mechanism of regulation (18). To gain a clear understanding of SOS activity and its regulation on membranes will require direct observations of individual SOS molecules functioning in a membrane environment.

Single-molecule SOS activity assay

We developed an assay platform to observe real-time single-molecule activity of SOS with Ras in a partitioned, supported membrane (Fig. 1B). H-Ras(C118S, 1 to 181) (referred to as Ras from here on) was linked to the membrane through maleimide coupling of Cys¹⁸¹ to supported lipid bilayers (SLBs), resulting in stably bound, laterally mobile Ras that is fully functional with respect to SOS activation (19, 20). We also used an H-Ras construct truncated at Cys¹⁸⁴ [H-Ras(C118S, 1 to 184)], which contains both native cysteine palmitoylation sites (181 and 184) in the hypervariable region (HVR), which we linked to lipids through maleimide chemistry. Membranes were composed primarily of 1- α -phosphatidylcholine (Egg, Chicken) (Egg-PC) with 2 to 3% anionic 1,2-dioleoyl-sn-glycero-3-phospho-L-serine lipids (DOPS) and in some cases PIP₂, which bind the PH domain on SOS and release autoinhibition (19). Molecules within the supported membrane were confined in micrometer-scale corrals by lithographically defined barriers to lateral mobility, prefabricated onto the underlying substrate (21). The barriers trapped membrane-tethered Ras and SOS (through its interactions with Ras and membrane lipids) within individual corrals. In each corral, the membrane remained entirely

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fluid, thus permitting SOS and Ras to diffuse freely while SOS processively catalyzed nucleotide exchange. Ras surface density was calibrated with fluorescence correlation spectroscopy (FCS) (22) and subsequently measured in each corral by means of epifluorescence imaging (fig. S1). Ras surface densities in these experiments ranged from several hundred to 1000 Ras molecules per square micrometer, corresponding to the broad Ras density range measured *in vivo* (8, 19). Mea-

surement of Ras lateral mobility with FCS and fluorescence recovery after photobleaching (FRAP) (fig. S2) ensured that fluid bilayers were partitioned by leak-free corrals.

SOS was introduced into the system from solution in a transient pulse traveling through the flow cell. We examined several SOS constructs derived from a truncated SOS containing the H, DH, PH, and C domains but lacking the C-terminal Grb2 binding domain (SOS-HDPC).

SOS binds to Ras on the membrane surface and, in the absence of free nucleotide, becomes trapped (3). After free SOS was rinsed from the system, unlabeled nucleotide was flowed in, and the exchange reaction commenced. By loading Ras with Atto488-labeled fluorescent nucleotide 2'/3'-O-(2-aminoethyl-carbamoyl)-guanosine-5'-diphosphate or -[(β,γ)-imido]triphosphate (Atto488-EDA-GDP or Atto488-EDA-GppNp, respectively; referred to as GDP-488 and GTP-488, respectively, from here on), catalytic exchange with nonfluorescent nucleotide from solution (GDP or GTP) could be directly observed as a local, corral-confined decrease of surface fluorescence. Constant flow during the exchange reaction ensured removal of unbound fluorescent nucleotides, allowing the reaction kinetics to be recorded by means of wide-field imaging of the fluorescence decay. Fluorescent and nonhydrolysable nucleotide analogs did not substantially perturb SOS activity (20).

Representative images taken during SOScat-catalyzed turnover showed a small percentage of active corrals (those with a SOS molecule) undergoing nucleotide exchange. These develop a clear negative contrast relative to inactive corrals (those without a SOS molecule) (Fig. 1C and movie S1). In all experiments, SOS concentrations were adjusted so that >95% of active corrals contained exactly one enzyme (fig. S3) (20). Total internal reflection fluorescence microscopy (TIRFM) of Atto647N-labeled SOScat (SOScat-647) revealed a clear correspondence between active dark corrals and individually confined SOScat enzymes (Fig. 1D and movie S2), which were laterally mobile within corrals (fig. S4) (20). However, it was not necessary to label SOS to run the assay. By imaging arrays of corrals on the surface, the exchange reactions of hundreds of individually confined enzymes were monitored in parallel (Fig. 1E). Quantitative analysis of fluorescence decays (fig. S5) (20) allowed individual kinetic traces of SOS activity to be analyzed (Fig. 1F). The total number of molecules tracked in an experimental run was similar to that in some bulk experiments (19), except that the individual contribution of every single molecule was observed.

SOScat enzymes were highly processive on the membrane surface. Hundreds to several thousand Ras could be activated by one SOScat molecule during a single membrane residency period (Fig. 1G). Comparable results for SOS activity were observed with both the single- (CI18S, 1 to 181) and double-anchored (CI18S, 1 to 184) Ras constructs (fig. S6). Because SOScat lacks any of the other membrane binding domains, this confirms that Ras alone is sufficient to stably recruit SOS to the membrane. A W729E point mutation in SOScat that abolishes Ras binding in the allosteric pocket (10, 19) inhibited processive activity (fig. S7). Bivalent interaction with Ras through both the catalytic and allosteric sites was required for sustained membrane localization of SOScat.

Dynamic heterogeneity of SOS activity

Kinetic traces of nucleotide exchange from individual SOS molecules revealed discrete transitions between well-defined catalytic states.

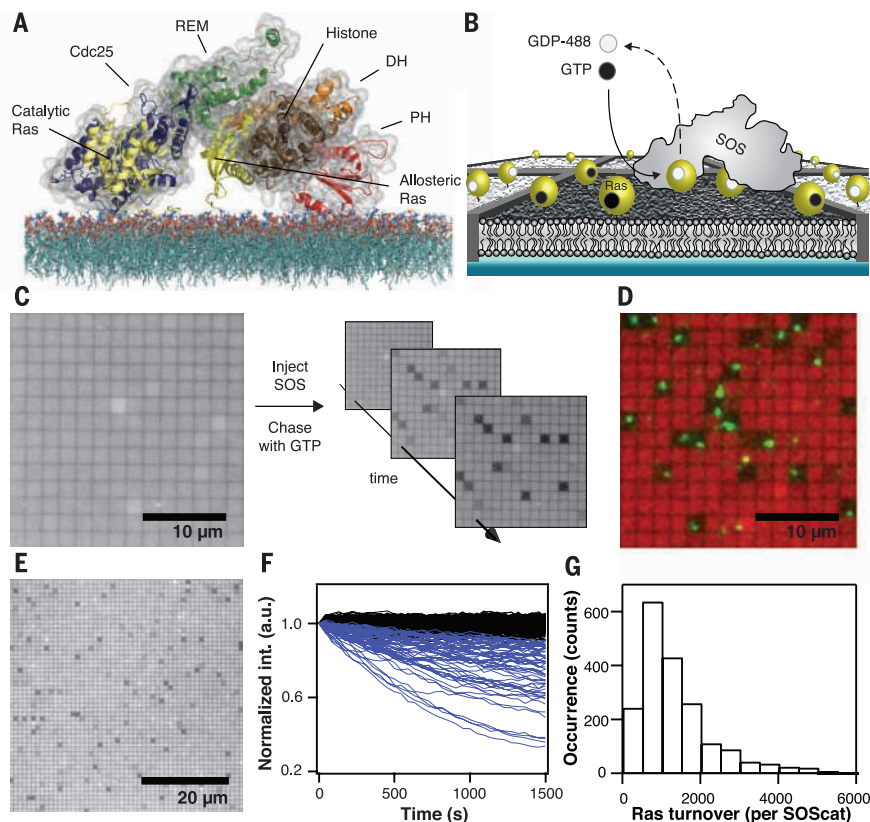


Fig. 1. Platform for single-enzyme kinetics. (A) Schematic showing the crystal structure and domain architecture of SOS-HDPC (surface and illustrated rendering) with Ras molecules (yellow, illustrated rendering) modeled onto the allosteric and catalytic sites, facing a lipid bilayer. SOS-HDPC is depicted in a sterically closed and auto-inhibited conformation. The actual on-membrane configuration is expected to be more open. The SOS-HDPC crystal structure, the HVR region of Ras, and the 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer structure are adapted from published works (20). (B) Scheme of the experimental setup. Nanofabricated chromium metal lines (10 nm high and 100 nm wide) partition a supported bilayer into micrometer-scale corrals, each containing lipid-anchored Ras loaded with fluorescent nucleotide at densities from hundreds to approximately a thousand molecules per square micrometer. When a single SOS engages Ras at the allosteric site, the catalytic site is free to turn over the remaining Ras in the corral, replacing fluorescent with nonfluorescent nucleotide and leading to a confined decrease of emission intensity. (C) Wide-field epifluorescence image of fluorescently loaded Ras before injection of SOS (left) shows no initial dark corrals. Injection of a pulse of SOS followed by continuous flow of nonfluorescent nucleotide (right) leads to enzymatic turnover in a subset of corrals. (D) False color overlay of fluorescently loaded Ras emission (red) and TIRFM image of fluorescently labeled SOScat-Atto647N (green) reveals colocalization of dark corrals and single SOScat enzymes. (E) Zoomed-out view showing a field of $1 \times 1 \mu\text{m}^2$ corrals with SOS activity. (F) Collection of single-corral kinetic traces from an array of SOScat turning over Ras-GTP, showing the percentage of fluorescent Ras in the corral as a function of time (blue traces). The black traces represent signals from empty corrals without enzymatic activity. (G) Histogram of total Ras-GTP-488 turnovers by individual SOScat enzymes. Scale bars, 10 μm (C) and (D); and 20 μm (E). Lipid composition (in molar percent): Egg-PC/MCC-PE/DOPS/TR-DHPE = 93.99/3/3/0.01.

Traces from two representative SOS molecules, each in its own corral, are plotted in Fig. 2A (more traces are provided in fig. S8). One molecule ran for more than 1200 s with a single catalytic rate (1.1 molecules/s). The other molecule shown started out with a similar rate, abruptly changed to 4 molecules/s at ~400 s, then abruptly changed back after another ~500 s. Using a change point algorithm (20, 23) to detect transitions revealed such events in 30% of traces for SOScat turning over Ras-GDP (fig. S9). Changes in turnover rate were not associated with changes in lateral diffusion of SOS (fig. S10). Because the transitions were discrete and the individual states exhibited well-defined kinetic rates, we suggest that these are the result of transitions between different stable configurations of the protein complex itself (SOScat with Ras bound in its allosteric site). Such conformational fluctuations have been traditionally observed on the microsecond to millisecond time scale (24–26), but longer-lived dynamic conformers exist in proteins (27), extending well into the minute time scale (supplementary text S2).

By identifying individual functional substates, we constructed the lifetime-weighted probability histogram of distinct catalytic rates sampled by hundreds of SOScat enzymes (Fig. 2B) (20). The histogram spans almost 2 orders of magnitude, with a broad peak at $\sim 1 \text{ s}^{-1}$ and a wide shoulder and tail of sparsely populated states extending toward higher rates. The histogram of apparent rates from inactive corrals, arising from intrinsic nucleotide release and photobleaching, represents the limit of resolution in this experiment (20). Even in a single catalytic state, stochastic variation resulting from the discrete nucleotide exchange events will lead to a real distribution of turnover rates over time and between enzymes. We estimated this intrinsic stochastic noise through simulations of a one-state system (supplementary text S3 and fig. S11). The overall distribution of SOScat turnover rates is much broader than either the experimental or intrinsic stochastic noise limits. However, it is likely that the assay does not resolve individual state peaks, giving instead the appearance of a broad continuous distribution.

SOS regulation through intradomain interaction

A key finding comes from a comparison of rate histograms for SOScat and SOS-HDPC (Fig. 2C and fig. S12). The N-terminal H, DH, and PH domains have a pronounced effect on the distribution of rates sampled by SOS. Whereas the peak of the rate distribution for SOS-HDPC overlaps with that of SOScat, the extended tail of molecules with faster rates is strongly suppressed, resulting in a sharper histogram. The N-terminal domains also dampen the frequency with which state transitions occur, dropping to 10% of kinetic traces with transitions for SOS-HDPC (fig. S9). These observations were made under conditions in which autoinhibition was previously thought to be released by interactions between the PH domain and anionic lipids (PS

or PIP₂ in these experiments) (10, 19, 28). However, we observed that the N-terminal domains still influence SOS activity by allosterically suppressing fluctuations that populate multiple high-activity states, which is consistent with a mechanism of conformational selection (supplementary text S2) (29–31). This represents a previously unknown layer of SOS regulation and emphasizes that not just the allosteric site, but also the N-terminal domains, communicate directly with the catalytic site.

The Noonan syndrome associated R552G SOS mutation leads to excessive activation of Ras in vivo (13) and increases the apparent rate of catalysis in bulk assays, but only when membranes are present (19). In vesicle assays, Ras activation is monitored as a function of the total amount of SOS in the system, but the fraction of SOS actually recruited to the membrane is unknown. The single-molecule rate histogram for SOS-HDPC(R552G) shows that the R552G mutation is not activating at the level of SOS specific activity (Fig. 2C) and results in a similar low-fluctuation frequency as seen for SOS-HDPC (fig. S9). Taken together with this observation, the accelerated turnover of Ras-GTP by SOS-HDPC(R552G) reported from vesicle assays (19) is likely to be a result of increased surface binding from solution. Abnormally high membrane recruitment of SOS-HDPC(R552G) may contribute to the pathogenic hyperactivation of Ras signaling in vivo as well.

Allosteric regulation of SOS

A second key observation is that the expected activation of SOS by Ras-GTP bound in its allo-

steric site is surprisingly weak over most of the range of the observed distribution of rates. Rate histograms for SOScat, SOS-HDPC, and SOS-HDPC(R552G) are shown in Fig. 3, A, B, and C, respectively. Although small differences in the distributions and most probable rates do exist, it is unlikely that these minor effects would be consequential in the context of a living cell. Some cellular signaling processes can be triggered by as little as a few individual ligand-receptor interactions (15–17). In such cases, only a small number of SOS molecules are likely to be involved as well. Variation of the ensemble mean (stochastic noise) becomes large when sample sizes become small, and thus, small differences in the mean rates become obscured in stochastic noise (supplementary text S4 and fig. S13).

However, the dynamics of SOS fluctuations do exhibit differences, depending on whether Ras-GTP or Ras-GDP is bound in the allosteric site. Whereas SOS accesses the rare, highly active states in either case, individual molecules linger for longer periods in these active states when interacting allosterically with Ras-GTP. This is made vivid if we examine the total Ras activation by individual SOS molecules. In Fig. 3, D, E, and F, each observed catalytic state is plotted on the basis of its specific activity and the Ras surface density on which it was observed. The total Ras turnover, equivalent to the number of Ras molecules activated by this particular SOS molecule while in this state, is represented by the size of the mark. Ras-GTP allosterically promotes high-activity long-lived states at the expense of low-activity shorter-lived states (figs. S14

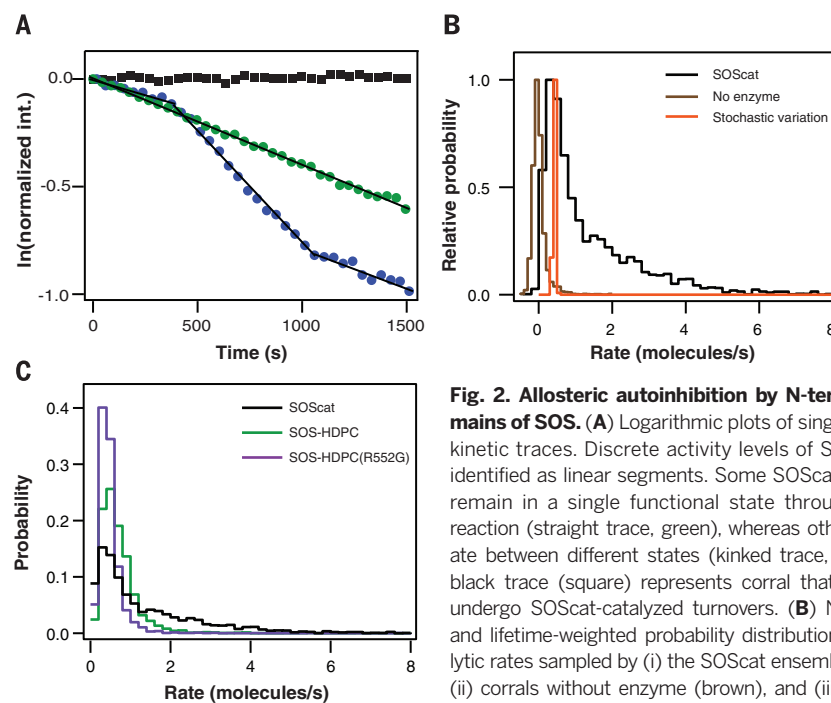


Fig. 2. Allosteric autoinhibition by N-terminal domains of SOS. (A) Logarithmic plots of single-enzyme kinetic traces. Discrete activity levels of SOScat are identified as linear segments. Some SOScat enzymes remain in a single functional state throughout the reaction (straight trace, green), whereas others fluctuate between different states (kinked trace, blue). The black trace (square) represents corral that does not undergo SOScat-catalyzed turnovers. (B) Normalized and lifetime-weighted probability distributions of catalytic rates sampled by (i) the SOScat ensemble (black), (ii) corrals without enzyme (brown), and (iii) modeled single-state enzyme stochastic variation (orange).

(C) Catalytic rate probability distributions from SOScat (black), SOS-HDPC (green), and SOS-HDPC (R552G) (purple). The N-terminal domains auto-inhibit SOS specific activity. A Noonan syndrome-associated point mutation in SOS-HDPC(R552G) (purple) does not relieve inhibition. Lipid composition (in molar percent): Egg-PC/MCC-PE/DOPS/TR-DHPE = 93.99/3/3/0.01.

and S15). In cells, these highly active individual molecules would produce all of the Ras they activate in the same location (supplementary text S5) and over a short time. This is especially consequential in a signaling context, in which GTPase-activating proteins (GAPs) catalyze the hydrolysis of GTP to GDP, thus inactivating Ras at a basal level. The effects of such GAP activity would render small bursts of Ras activation inconsequential but may not keep pace with the highly active molecules. In such a situation, the average activity of the entire SOS ensemble is relatively unimportant because the rare, highly active individuals dominate the overall system behavior. Furthermore, the important functional consequences of feedback regulation of SOS by Ras-GTP observed in computer simulations and cellular experiments (8) could well be mediated by the long-lived active states that allosteric regulation by Ras-GTP enables but that by Ras-GDP does not. Ras reportedly forms dimers on membrane surfaces (32). Such dimers could potentially affect SOS activity and, depending on the two-dimensional dimerization affinity on membrane surfaces, could contribute to the mild Ras density-dependence of SOS activity seen in Fig. 3D.

Effect of fluctuation dynamics on the signaling network

To explore the general feasibility of rate fluctuations that provide a mechanism of allosteric

regulation, we conducted a series of stochastic simulations, beginning with a simple enzymatic system that does not account for feedback regulation (Fig. 4, A and B). The simple system demonstrates the functional importance of rate fluctuations and the existence of long-lived active states. We consider a substrate, S, that is converted to its active state, S*, by an enzyme E, which can sample different possible states of activity (supplementary text S6 and fig. S16). A deactivating enzyme (analog of RasGAP) can convert S* to S. We first carried out Gillespie simulations (33) with single enzymes, with different trajectories corresponding to different choices of the enzyme activity and switching rates. In one set of trajectories, the enzymes were allowed to sample only low-activity states (allosteric Ras-GDP-bound SOS), whereas in another set, an additional highly active state was sampled (allosteric Ras-GTP); in another set of simulations, the enzymes sampled identical enzymatic rate distributions but transitioned between catalytic states at different rates. Our simulations demonstrate that placing additional weight in the highly active tail of the distribution or having enzymes with long-lived states can lead to fast threshold-crossing by a subset of the enzymes, whereas a collection of enzymes all operating at an increased but average catalytic rate are unable to support activation (Fig. 4A). We also made simulations in which the rates of a number of enzymes in a single reaction group [such as a cell or a localized mem-

brane signaling cluster (34)] were chosen from the same distribution, but their rates of switching between states were different. Histograms of simulation results for many such reaction groups showed that for small numbers of enzymes (analog of weak stimulation), slower switching rates can enable some groups to become activated, whereas activity at the average rate would not (Fig. 4B).

We also investigated a coarse-grained model of lymphocyte SOS signaling, which has been used to interpret bimodal early signaling events in lymphocytes (supplementary text S7) (8). This model includes feedback regulation through Ras-GTP and the interplay between SOS and the RasGEF Ras guanyl nucleotide-releasing protein (RasGRP), as well as the signal attenuation through GAP activity (8). The resulting histograms of cellular Ras-GTP levels are shown in Fig. 4C, each containing thousands of simulated cells. The existence of stochastic activity fluctuations between long-lived states in the simulated SOS-like GEF shifted the regime of bimodal Ras-GTP signaling (gray-shaded histograms) and broadened the ability of the network to exhibit bistability. This effect propagated through the network as a whole. Bimodality resulting from static SOS (Fig. 4D, red lines) or fluctuating SOS (Fig. 4D, blue lines) differed across the SOS and RasGRP concentration matrix (Fig. 4D). Gray-shaded histograms highlight regions of the parameter space where only fluctuating SOS enzymes gave rise to bimodal Ras-GTP distributions, illustrating how the threshold of the overall signaling network is fine-tuned by stochastic rate fluctuations of a single constituent enzyme—in this case, SOS. Various models have been advanced to explain how Ras-GTP (35, 36) and other cellular signals (37, 38) are spatiotemporally shaped to process information, for example, for analog or digital response to stimuli. Stochastic rate fluctuations, as reported here for SOS, constitute another mechanism for tuning cellular signaling dynamics (39).

Dynamic heterogeneity has been reported for multiple enzymes (40–42). However, the duration of dynamic SOS states we observed [several minutes (Fig. 3B)] is longer than that reported for other enzyme systems [up to tens of seconds (41, 42)]. The SOScat fluctuation behavior shows that dynamic conformational modes in signaling enzymes may extend well into time scales comparable with receptor signaling processes (37, 39). Evolutionary pressure is required to evolve and maintain increased enzymatic efficiency (43), so high-activity states are likely to be biologically important. SOS is the key enzyme involved in bimodal switching of Ras-GTP levels during T cell receptor-triggering in T cells (8, 44). It is certainly possible that rare high-activity states could function in threshold-crossing and bistability, leading to robust Ras-GTP positive-feedback activation of SOS and robust response to antigen.

More fundamentally, we developed a single-molecule enzymatic assay that enables detailed observation of the activation of membrane-linked

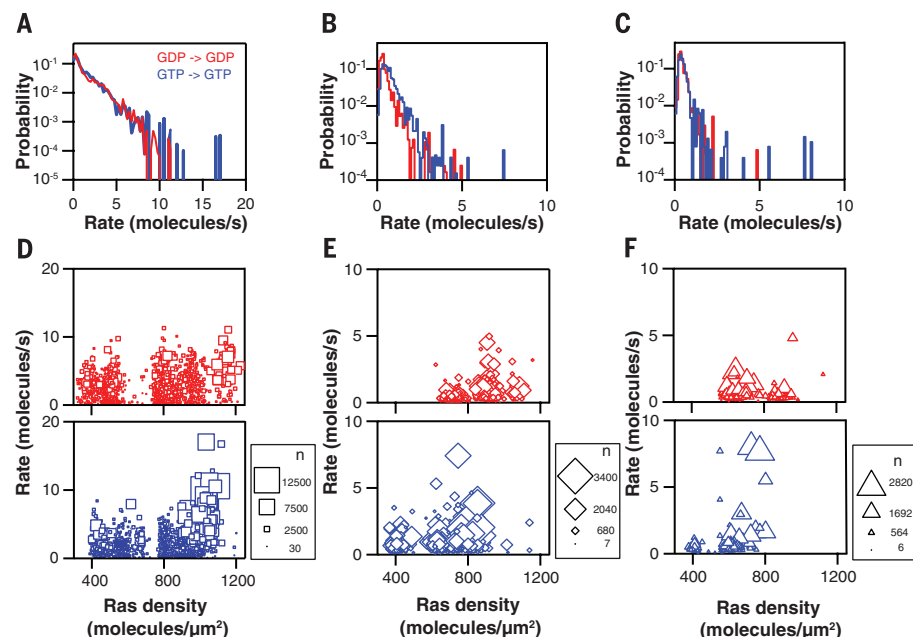


Fig. 3. Nucleotide specificity of allosteric activation of SOS. (A to C) The nucleotide-dependent rate distributions for (A) SOScat, (B) SOS-HDPC, and (C) SOS-HDPC(R552G) on a logarithmic scale. For all constructs, Ras-GTP bound in the allosteric site of SOS (blue traces) has a small activating effect relative to Ras-GDP in the allosteric site (red traces). (D to F) Scatterplots of SOS turnover rate as a function of Ras surface density for (D) SOScat, (E) SOS-HDPC, and (F) SOS-HDPC(R552G). Each point in the scatterplots represents an observed catalytic state, and the area of the point represents the number of Ras turned over by that catalytic state. For all constructs, Ras-GTP bound in the allosteric site of SOS (blue points) results in longer-lived high-activity states capable of highly processive catalysis. Lipid composition (in molar percent): Egg-PC/MCC-PE/DOPS/TR-DHPE = 93.99/3/3/0.01.

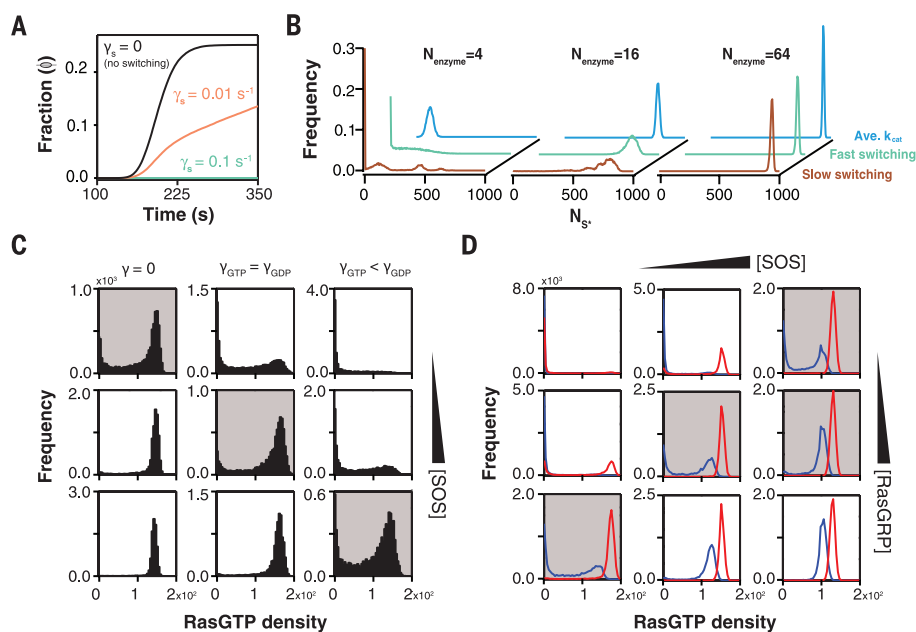


Fig. 4. Modeling effects of stochastic transitions between long-lived enzymatic states by using a minimal signaling model or broader network model. (A) Fraction of trajectories (ϕ) reaching a threshold amount (N_{S^*}) of product S^* during activation by single GEF-like enzymes fluctuating with switching frequencies (γ_S). GAP-like enzymes simultaneously deactivate S^* to S at a fixed rate. The ensembles of enzymes represented by the three traces have identical mean activity levels and differ only in the rate with which enzymes fluctuate between activity states (details of the model are provided in supplementary text S6). Enzymes with a fast switching rate (green trace) do not reach the signaling threshold, whereas enzymes with a lower switching rate (orange trace) do. Enzymes that do not switch between states on the time scale of the simulation show the most rapid activation and threshold crossing. Additionally, no threshold crossing is observed for static enzymes operating at the average catalytic rate. **(B)** Probability distributions of (N_{S^*}) for various ensemble sizes ($N_{\text{enzyme}} = 4, 16, \text{ or } 64$), with slow switching (brown trace, $\gamma_S = 10^{-3}$), fast switching (green trace, $\gamma_S = 10^{-1}$), or enzymes operating only at the average catalytic rate (blue trace). In all cases, the average catalytic rate is the same. At low (4) and intermediate (16) copy number, the activation pattern depends strongly on the switching rate. At low copy number, slow switching gives rise to a population of highly activating enzymes (resulting in more than 400 S^*). **(C)** Distributions of Ras-GTP resulting from coarse-grained modeling of the Ras-SOS signaling network (details of the model are provided in supplementary text S7). Each histogram contains 8000 modeled cells. Left column shows simulations without catalytic rate fluctuations, $\gamma_S = 0$. Middle column shows simulations with catalytic rate fluctuations for which the switching rate of SOS with Ras-GTP or Ras-GDP allosterically bound is the same: $\gamma_{\text{GTP}} = \gamma_{\text{GDP}}$. Right column shows simulations in which SOS with Ras-GTP allosterically bound switches more slowly than did SOS with Ras-GDP allosterically bound: $\gamma_{\text{GTP}} < \gamma_{\text{GDP}}$. For each switching condition, the Ras-GTP distribution is plotted for increasing cellular levels of SOS. **(D)** Distributions of Ras-GTP resulting from static SOS (red traces) or fluctuating SOS (blue traces). The histograms span a matrix of cellular levels of the two RasGEFs SOS and RasGRP. Conditions in which only fluctuating SOS gives rise to a bimodal Ras-GTP distribution are shaded gray.

GTPases by GEFs. This system is generalizable to a broad class of such interactions. We anticipate that fluctuations and distributions of rates may well prove to be just as important, if not more so, than ensemble averages in the behavior of cellular signaling networks. The single-molecule membrane surface assays introduced here render such phenomena accessible to quantitative experimental investigation.

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SUPPLEMENTARY MATERIALS

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REPORTS

BILAYER GRAPHENE

Electron-hole asymmetric integer and fractional quantum Hall effect in bilayer graphene

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The nature of fractional quantum Hall (FQH) states is determined by the interplay between the Coulomb interaction and the symmetries of the system. The distinct combination of spin, valley, and orbital degeneracies in bilayer graphene is predicted to produce an unusual and tunable sequence of FQH states. Here, we present local electronic compressibility measurements of the FQH effect in the lowest Landau level of bilayer graphene. We observe incompressible FQH states at filling factors $\nu = 2p + 2/3$, with hints of additional states appearing at $\nu = 2p + 3/5$, where $p = -2, -1, 0$, and 1 . This sequence breaks particle-hole symmetry and obeys a $\nu \rightarrow \nu + 2$ symmetry, which highlights the importance of the orbital degeneracy for many-body states in bilayer graphene.

The charge carriers in bilayer graphene obey an electron-hole symmetric dispersion at zero magnetic field. Application of a perpendicular magnetic field B breaks this dispersion into energy bands known as Landau levels (LLs). In addition to the standard spin and valley degeneracy found in monolayer graphene, the $N = 0$ and 1 orbital states in bilayer graphene are also degenerate and occur at zero energy (1). This results in a sequence of single-particle quantum Hall states at filling factor $\nu = 4M$, where M is a nonzero integer (2).

When the disorder is sufficiently low, the eightfold degeneracy of the lowest LL is lifted by electron-electron interactions, which results in quantum Hall states at all integer filling factors (3, 4). External electric and magnetic fields can be used to induce transitions between quantum Hall ground states with different spin and valley orders (5–8). For example, the $\nu = 0$ state, which is a canted antiferromagnet at large perpendicular magnetic field, can be tuned into a ferromagnetic state by a large parallel magnetic field, or a layer-polarized state under large perpendicular electric field (5–11). The order in which orbital states are filled remains an open question, with suggestions of full polarization or orbitally coherent states, depending on system parameters (12–16). The interplay between externally applied fields and intrinsic electron-electron interactions, both of which break the degeneracies of bilayer graphene, produces a rich phase diagram.

Knowledge of the ground state at integer filling factors is especially important for investigating the physics of partially filled LLs, where, in exceptionally clean samples, the charge carriers condense into fractional quantum Hall (FQH) states. The above-mentioned degrees of freedom, as well as the strong screening of the Coulomb interaction in bilayer graphene, are expected to result in an interesting sequence of FQH states in the lowest LL (16–21). Partial breaking of the SU(4) symmetry in monolayer graphene has already resulted in sequences of FQH states with multiple missing fractions (22–26).

The few FQH states reported in bilayer graphene thus far include hints of a $\nu = 1/3$ state (27) and robust FQH states at $\nu = -1/2$ and $-4/3$ in

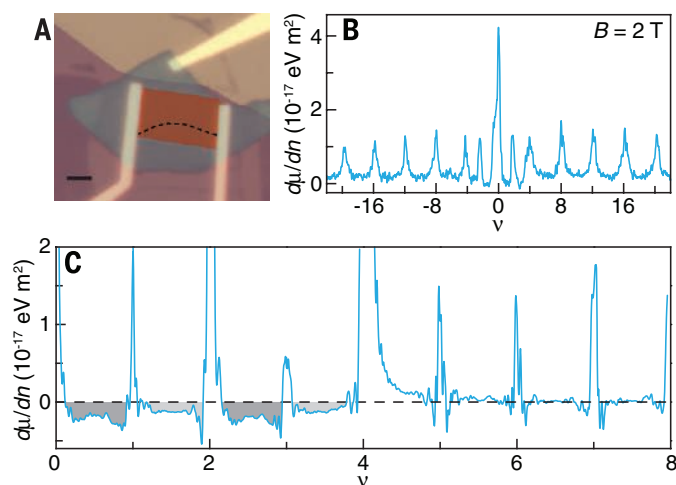
suspended samples (28). Here, we report local compressibility measurements, performed using a scanning single-electron transistor (29, 30), of a bilayer graphene device fabricated on hexagonal boron nitride (h-BN). An optical image of the contacted device is shown in Fig. 1A.

Figure 1B shows a measurement of the inverse compressibility $d\mu/dn$ as a function of filling factor at $B = 2$ T. Incompressible features are present at all nonzero multiples of $\nu = 4$, as expected for bilayer graphene. The full width at half maximum of the $\nu = 4$ peak provides a measure of the disorder in the system and is on the order of 10^{10} cm^{-2} , similar to that observed in suspended bilayers (3, 31). Broken-symmetry states at $\nu = 0$ and ± 2 are also visible at $B = 2$ T, which further indicates the cleanliness of the sample. In Fig. 1B, the inverse compressibility appears more negative for $|\nu| < 4$ than in higher LLs, and we explore this behavior further by averaging the inverse compressibility between 8 and 11.5 T, which reduces fluctuations caused by localized states (Fig. 1C). The background inverse compressibility between integer quantum Hall states is close to zero at $4 < \nu < 8$ but is markedly more negative for $0 < \nu < 4$, qualitatively consistent with observations in the lowest LL in monolayer graphene (26, 32, 33).

Even within the lowest LL, the background inverse compressibility has two different characteristic shapes. It is more negative and less flat when increasing the filling factor from an even value than from an odd value. One might expect that as states are filled with electrons from an even filling factor, electron-electron interactions break the degeneracy between the $N = 0$ and 1 orbital LLs, so that only the $N = 0$ LL is occupied. When states are filled with electrons from an odd filling factor, the $N = 0$ LL is already full, so electrons start to occupy the $N = 1$ LL. The more negative background inverse compressibility between $\nu = 0$ and 1 and between $\nu = 2$ and 3 points to the presence of less screening or more electron-electron correlations in the

Fig. 1. Sample image and characterization.

(A) Optical image of the device with colored overlays showing the graphite (blue), boron nitride (purple), and monolayer-bilayer graphene hybrid (red). The dashed line marks the interface between monolayer and bilayer graphene. Scale bar, $2 \mu\text{m}$. (B) Inverse compressibility $d\mu/dn$ as a function of filling factor ν at magnetic field $B = 2$ T. (C) Average inverse compressibility between $B = 8$ and 11.5 T as a function of filling factor after current annealing to 4 V. Shaded areas indicate regions of more negative background inverse compressibility.



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$N = 0$ LL when compared with the $N = 1$ LL with an underlying filled $N = 0$ LL.

Figure 2, A and C, shows the inverse compressibility as a function of filling factor and magnetic field after we further cleaned the sample by current annealing. Quantum Hall states appear as vertical features when plotted in this form, whereas localized states, which occur at a constant density offset from their parent states, curve as the magnetic field is changed (26, 34). We can then unambiguously identify the incompressible states that appear at $\nu = -10/3, -4/3, 2/3$, and $8/3$ as FQH states. These states follow a $\nu = 2p + 2/3$ sequence, where $p = -2, -1, 0$, and 1 . Above 10 T, we also see evidence of developing states at $\nu = -17/5, -7/5, 3/5$, and $13/5$, which follow a similar $\nu = 2p + 3/5$ sequence. The FQH states closer to the charge-neutrality point are more incompressible than those at higher filling factors, and they persist to fairly low magnetic fields, with the last hints disappearing around 6 T.

The line plots in Fig. 2, B and D, show the average inverse compressibility in each filling factor range from 7.9 to 11.9 T. The plots highlight the FQH states identified above, as well as the different behaviors exhibited by the inverse compressibility, which shows especially strong divergences as the filling factor is increased from $\nu = 0$ and 2 . Similar to $\nu > 0$, the background inverse compressibility at negative filling factors displays an even-odd effect with more negative values when increasing the filling factor from an even integer. The FQH states coincide with areas of more negative background inverse compressibility, consistent with its attribution to Coulomb interactions (32, 35). Despite theoretical predictions of robust FQH states in the $N = 2$ LL and experimental hints in other samples

(8), we do not observe any FQH states between $|\nu| = 4$ and 8 .

Notably, the observed sequence of FQH states and the background inverse compressibility pattern break particle-hole symmetry and instead follow a $\nu \rightarrow \nu + 2$ pattern. The $\nu \rightarrow \nu + 2$ symmetry indicates that the orbital degeneracy is playing an important role. This symmetry was predicted based on a model that incorporates the strong screening and LL mixing present in the lowest LL of bilayer graphene (16). The absence of FQH states between $\nu = -3$ and -2 or in the intervals connected to $-3 < \nu < -2$ by $\nu \rightarrow \nu + 2$ symmetry suggests a difference in electron-electron interactions between partial filling when both the $N = 0$ and 1 LLs are empty and partial filling of the $N = 1$ LL when the $N = 0$ LL is full. The increased LL mixing present when the $N = 0$ LL is full (36) may be weakening the strength of FQH states in the $N = 1$ LL.

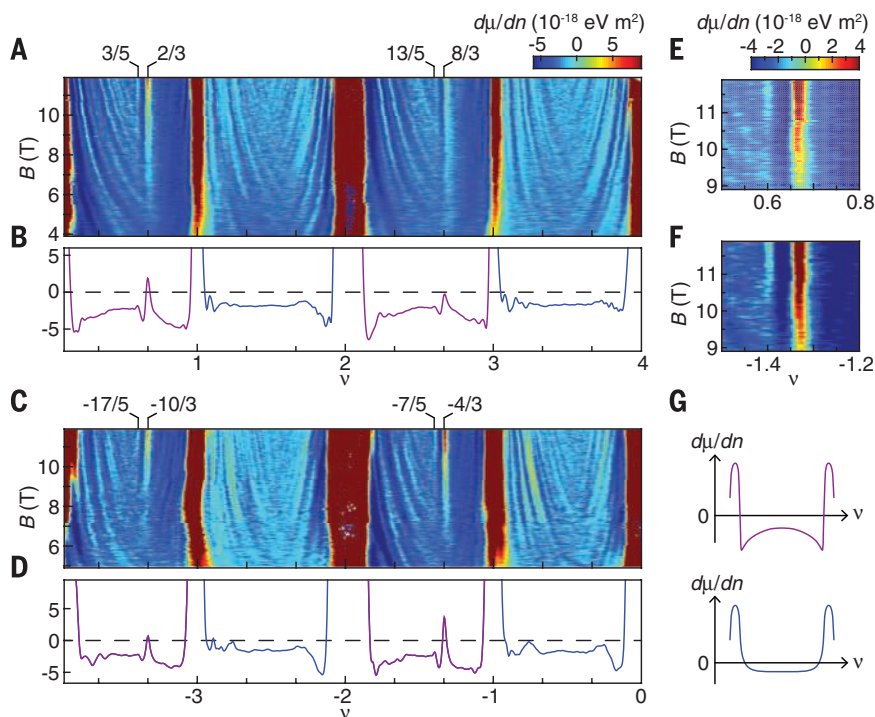
Our observed FQH sequence also suggests possible orbital polarization of the FQH states. The FQH states we observe at $\nu = 2p + 2/3$ could be singlet-like states of $N = 0$ and 1 orbitals or could arise from a $\nu = 2/3$ state with full orbital polarization. The next strongest FQH states that we observe occur at $\nu = 2p + 3/5$, which must have some nonzero orbital polarization. The strongest FQH states at multiples of $\nu = 1/5$ do not have even numerators, in contrast to recent theoretical predictions (16).

The FQH states that we observe are different from those seen in previous experiments on bilayer graphene, which may point to different patterns of symmetry-breaking in the different systems. In (28), FQH states at $\nu = -4/3$ and $-1/2$ were observed, with hints of additional features at $\nu = -8/5$ and $-2/3$; the only FQH state seen in

both devices is $\nu = -4/3$. It is also possible that the effective interactions present in the two samples may be different because of the differences in screening between a suspended bilayer and a bilayer on a substrate. The fact that different sample preparations result in different FQH states could be a sign of the theoretically predicted tunability of the FQH effect in bilayer graphene (17, 19, 21). Applying a perpendicular electric and/or a parallel magnetic field to the sample may provide insight into the conditions under which different FQH states are favored. We can contrast this with monolayer graphene, in which both suspended and substrate-supported samples have shown similar sequences of FQH states (22–26, 37). Though phase transitions of FQH states were observed (38) in monolayers, these involved changes in only the spin and/or valley polarization; the sequence of observed FQH states did not change. In bilayer graphene, however, it appears that the sample geometry and/or substrate play an important role in determining the relative strengths of various incompressible FQH states.

We can integrate the inverse compressibility with respect to density to obtain the energy cost of adding an electron to the system (26). This quantity must be divided by the quasi-particle charge associated with each state to determine the corresponding energy gap Δ_ν . The most likely quasi-particle charge for states at multiples of $\nu = 1/3$ is $e/3$ (where e is the charge of an electron), but the nature of the FQH states in bilayer graphene is not yet fully understood, so we plot the extracted steps in chemical potential $\Delta\mu_\nu$ in Fig. 3A. For $\nu = -4/3$ and $2/3$, $\Delta\mu_\nu$ is ~ 0.75 and ~ 0.6 meV, respectively, at $B = 12$ T. Assuming a quasi-particle charge of $e/3$, the energy gap we

Fig. 2. Fractional quantum Hall states in bilayer graphene. (A and C) Inverse compressibility as a function of filling factor and magnetic field. The color scales are the same in both panels. (B and D) Average inverse compressibility between $B = 7.9$ and 11.9 T as a function of filling factor. Colors indicate regions of similar behavior in the background inverse compressibility. (E and F) Inverse compressibility as a function of filling factor and magnetic field near $\nu = -7/5$ and $3/5$. (G) Diagram highlighting the differences in background inverse compressibility between $\nu = 2p$ and $\nu = 2p + 1$ in purple and $\nu = 2p + 1$ and $\nu = 2p$ in blue.



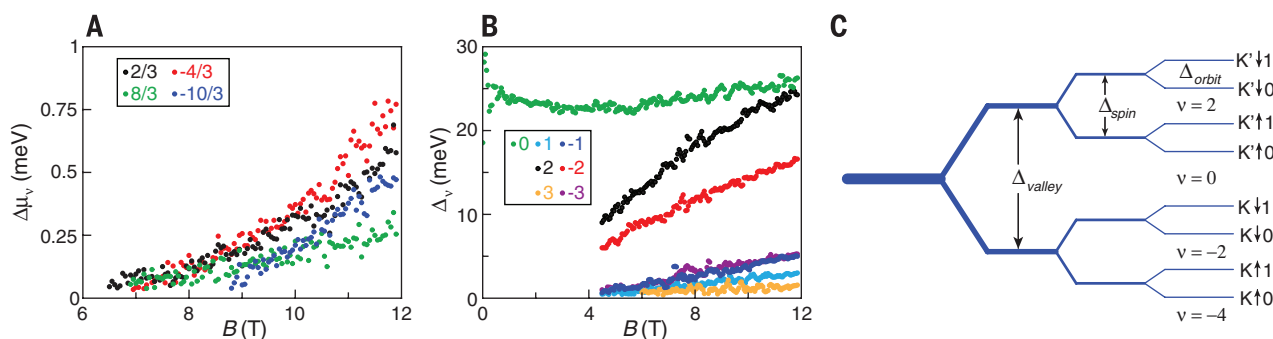


Fig. 3. Steps in chemical potential. (A) Steps in chemical potential of the fractional quantum Hall states as a function of magnetic field. (B) Energy gaps of the integer broken-symmetry states in the lowest LL. (C) Diagram showing the order of symmetry-breaking in the sample. K and K' denote the valleys of bilayer graphene.

find at $\nu = -4/3$ is comparable with, if somewhat larger than, that found in (28) at similar magnetic fields. The gaps of FQH states further away from charge neutrality are smaller; $\Delta\mu_{8/3}$ and $\Delta\mu_{-10/3}$ are only ~ 0.5 and ~ 0.3 meV, respectively, at $B = 12$ T. All of the extracted gaps increase monotonically with B . The gaps appear to scale approximately linearly or, in some cases, even superlinearly with field, although we cannot explicitly rule out a $\sqrt{B}$ -dependence of the gaps at $\nu = 8/3$ and $-10/3$. Previous measurements of broken-symmetry integer states in suspended bilayers revealed a linear B dependence of the gaps, which was attributed to LL mixing (31, 39). The magnitude of the FQH energy gaps is probably sensitive to the details of disorder in the system and perhaps also to the ratio between magnetic length and sample-gate distance.

The energy gaps of the integer filling factors $|\nu| < 4$ are shown in Fig. 3B. All of the gaps increase with B , except for $\nu = 0$, which is fairly constant around 23 to 25 meV over almost the full range in the magnetic field. Around 4 T, the gap dips slightly before increasing again at $B = 0$ T. The size of the gap and its persistence to zero field lead us to conclude that the ground state at $\nu = 0$ is layer-polarized. If we assume that the $\nu \rightarrow \nu + 2$ symmetry arises from the orbital degree of freedom, we can fully determine the sequence of symmetry-breaking in the sample (Fig. 3C): valley polarization is first maximized, then spin polarization, and finally orbital polarization. The large valley polarization in our sample relative to other bilayer devices may be caused by interactions with the substrate (40). Large band gaps have been observed in monolayer graphene samples on h-BN with a proximal gate, which have been attributed to the breaking of sublattice symmetry by h-BN or screening from the nearby metal (37, 41, 42). It is also possible that the difference in distance between the top layer to the graphite gate and the bottom layer to the graphite gate is creating a potential difference in the two layers (43), or that the different environments experienced by each layer play a role. Even if the $\nu = 0$ gap is caused by single-particle effects, its constancy over our entire field range is somewhat surprising because both the potential difference be-

tween the layers and the Coulomb energy are expected to contribute to the gap (44).

Local measurement allows us to probe multiple locations on the sample, and although we do observe fluctuations in the strengths of the broken-symmetry and FQH states, the overarching pattern of FQH states is consistent across the entire sample (fig. S1) and also did not change with current annealing (fig. S2). The electron-hole asymmetric sequence of FQH states can therefore be attributed to the intrinsic properties of bilayer graphene, rather than disorder or other local effects. The observation of an unconventional sequence of FQH states in bilayer graphene indicates the importance of its underlying symmetries and opens avenues for exploring the nature and tunability of the FQH effect.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
Figs. S1 to S3
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BILAYER GRAPHENE

Chemical potential and quantum Hall ferromagnetism in bilayer graphene

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Bilayer graphene has a distinctive electronic structure influenced by a complex interplay between various degrees of freedom. We probed its chemical potential using double bilayer graphene heterostructures, separated by a hexagonal boron nitride dielectric. The chemical potential has a nonlinear carrier density dependence and bears signatures of electron-electron interactions. The data allowed a direct measurement of the electric field-induced bandgap at zero magnetic field, the orbital Landau level (LL) energies, and the broken-symmetry quantum Hall state gaps at high magnetic fields. We observe spin-to-valley polarized transitions for all half-filled LLs, as well as emerging phases at filling factors $\nu = 0$ and $\nu = \pm 2$. Furthermore, the data reveal interaction-driven negative compressibility and electron-hole asymmetry in $N = 0, 1$ LLs.

The electronic structure of bilayer graphene has a bandgap and energy-momentum dispersion that can be tuned by an applied transverse electric field (*1–4*). In high magnetic fields, electron-electron interaction coupled with the spin and valley degrees of freedom can lead to a diverse set of quantum Hall states (QHSs) (*1–8*). Thermodynamic measurements of the chemical potential or the density of states are fundamental to understanding the electronic properties of bilayer graphene (*9–12*). The density of states of bilayer graphene extracted from compressibility data (*9, 10*) shows modulations associated with fourfold, spin- and valley-degenerate Landau levels (LLs), whereas local compressibility measurements in single-gated suspended bilayer graphene samples reveal broken-symmetry QHSs (*11*).

Here, we studied samples consisting of two Bernal-stacked bilayer graphene separated by a thin hexagonal boron nitride (hBN) (Fig. 1A). The samples are fabricated with a sequence of mechanical exfoliation, dry-transfer, and alignment with a preexisting device, similarly to the method described in (*13, 14*). The bottom bilayer graphene is supported by a thick (30 to 50 nm) hBN substrate, mechanically exfoliated on a SiO₂/Si substrate. A relatively thin (2 to 6 nm) hBN layer is then transferred and aligned with the bottom bilayer, followed by the top bilayer graphene transfer. Each bilayer graphene is patterned into a multiterminal Hall bar, and an etch-through of the two bilayers ensures that the Hall bar edges are aligned (Fig. 1B, inset). The bilayer resistances are measured as a function of back-gate (V_{BG}) and interbilayer bias applied to the top bilayer (V_{TL}) by means of small-signal, low-frequency lock-in techniques. Three samples, labeled #1,

#2, and #3, were investigated in this study. The bottom bilayers in all the samples have mobilities ranging between 100,000 and 290,000 cm²/V-s, whereas the top bilayers have lower mobilities, ranging between 3400 and 6500 cm²/V-s.

The top (n_T) and bottom (n_B) bilayer carrier densities depend on V_{BG} and V_{TL} as (*15*):

$$eV_{BG} = e^2(n_B + n_T)/C_{BG} + \mu_B \quad (1)$$

$$eV_{TL} = -e^2n_T/C_{int} - \mu_T + \mu_B \quad (2)$$

Here, C_{BG} and C_{int} represent the bottom and interbilayer dielectric capacitances, whereas μ_T and μ_B represent the chemical potentials (Fermi energies) of the top and bottom bilayers, respectively; e is the electron charge. We note that μ and n are positive (negative) for electrons (holes), and V_{BG} and V_{TL} in Eqs. 1 and 2 are referenced with the bias values at which both bilayers are charge neutral, i.e., at the double neutrality point (DNP). The externally applied transverse electric field (E) across the bottom bilayer can be written as

$$E = en_B/2\epsilon_0 + en_T/\epsilon_0 + E_0 \quad (3)$$

where ϵ_0 is the vacuum permittivity, n_B and n_T are determined by Eqs. 1 and 2, and E_0 is an additive constant that allows for a finite E -field at the DNP, associated with an unintentional doping of the top bilayer.

The measured bottom bilayer density and resistance dependence on V_{BG} and V_{TL} (Fig. 1C) is similar to that of a dual-gated bilayer (*16–18*), where the charge neutrality point has a linear dependence on V_{BG} and V_{TL} , with a slope controlled by C_{BG} and C_{int} . Along the charge neutrality line of the bottom bilayer, the resistance is minimum at $E = 0$ and increases with the E -field due to the E -field-induced bandgap of bilayer graphene. By comparison to the bottom

bilayer, the top bilayer graphene resistance (Fig. 1B) has a weak dependence on V_{BG} because of the screening by the bottom bilayer and is controlled primarily by V_{TL} . Notably, setting $n_T = 0$ in Eq. 2 yields $eV_{TL} = \mu_B$, which implies that the interbilayer bias required to bring the top bilayer to charge neutrality, marked by dashed lines in Fig. 1, B and C, is simply the chemical potential of the bottom bilayer (Fig. 1D) in units of eV (*15*). Using Eq. 1, the n_B values along the top bilayer neutrality line (dashed lines in Figs. 1, B and C) are $n_B = C_{BG} \cdot (V_{BG} - V_{TL})/e$. Consequently, the bottom bilayer chemical potential can be mapped as a function of its carrier density.

Figure 2A shows the bottom bilayer chemical potential (μ) versus density (n) determined as described above. The finite doping of the top bilayer at $V_{TL} = V_{BG} = 0$ V in our samples leads to a finite E -field across the bottom bilayer at the DNP: $E_0 = 0.54, 0.21$, and 0.46 V/nm for samples #1, #2, and #3, respectively. From Eqs. 1 to 3, it follows that $E_0 = C_{BG} \cdot \Delta V_{BG}/\epsilon_0$, where ΔV_{BG} is the difference between the back-gate bias at the DNP and at $n = 0$ and $E = 0$ point (Fig. 1C). The μ versus n for the lowest-energy band in bilayer graphene calculated within a tight binding approximation (*3*) for $E = 0$ and $E = 0.54$ V/nm are included for comparison (dashed lines), using a noninteracting in-plane velocity $v = 8.4 \times 10^5$ m/s and interlayer hopping $\gamma_1 = 0.34$ eV (*19*), and neglecting trigonal warping. Because of the interaction-induced renormalization of electron energies (*20*), the measured $|\mu|$ values are, particularly at high densities, larger than the results of the band calculations. The nonlinear μ versus n dependence indicates a strongly nonparabolic energy-momentum dispersion (*3, 4*). Such a dispersion would lead to a density-dependent effective mass, as observed in (*21*). This is confirmed in Fig. 2B, which shows a nonmonotonic dependence of the effective mass m^* on density n , extracted from Fig. 2A data using $m^* = (\pi\hbar^2/2)(d\mu/dn)^{-1}$. At low densities, m^* increases with the E -field and shows a divergence as a function of n near charge neutrality. The measured μ value also shows a clear discontinuity as n changes sign, revealing a gap Δ (Fig. 2C) associated with the E -field-induced band-gap in bilayer graphene (*3, 4*).

In a perpendicular magnetic field ($B = 14$ T), sample #1 shows clear minima of the longitudinal resistivity ρ_{xx} (Fig. 3A), indicating QHSs at all integer filling factors (ν) up to $|\nu| = 15$; the $\nu = 0$ QHS is marked by a ρ_{xx} maximum. The QHSs at $\nu \neq \pm 4, 8, 12, \dots$ stabilized by the spin and valley degeneracy lifting induced by the interaction-enhanced Zeeman splitting (*6*) and the E -field, respectively, undergo transitions at finite E -field values as a result of the interplay between the LL spin and valley splitting. Specifically, the $\nu = -1, -3, -5, -7, -9, -11$ (odd filling) QHSs are absent at $E = 0$ and emerge as an E -field is applied. By contrast, the half fillings $\nu = 0, -6, -10$ of the spin- and valley-degenerate LLs are present at $E = 0$ and collapse at finite E -field values. The interplay between the spin and valley splitting explains the transitions at $\nu = -6, -10, -14$ at a finite E -field (Fig. 3A and supplementary text).

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Figure 3B and fig. S3 show a strong $\nu = 0$ QHS at $E = 0$, as well as at high E -field, consistent with (17, 18). Both theory and experiment (7, 22) indicate that the insulating $\nu = 0$ QHS in

the proximity of $E = 0$ is described by a canted antiferromagnetic (CAF) ground state, where electrons in different valleys have opposite in-plane spin orientation and a net out-of-plane

spin polarization; the $\nu = 0$ state at high E -field is valley (layer) polarized. In Fig. 3B, the insulating ρ_{xx} at $\nu = 0$ collapses at two distinct E -field values, rather than one (17, 18), indicating two distinct transitions and the observation of an intermediate phase in between the CAF and layer-polarized phases. The data can be qualitatively understood using the tight-binding LL energy diagram (Fig. 3C) (3). In this picture, the energies of LL with orbital index $N = 0, 1$ have a different dependence on the E -field, which explains the two distinct transitions at $\nu = 0$, as well as the emergence of $\nu = \pm 1$ and ± 3 QHSs at a finite E -field. When electron-electron interactions are included (8), the intermediate $\nu = 0$ QHS between the CAF and layer-polarized phases is found to be a more subtle spin-layer coherent phase, where LLs with the same N but different spin and valley degrees of freedom, e.g., solid red (orange) and dashed dark (light) blue in Fig. 3C, form coherent superpositions. In the single-particle picture, at $\nu = 0$, electrons in the $N = 0$, spin down, top-layer LL (solid red line in Fig. 3C) should move to the $N = 0$, spin up, bottom-layer LL (dashed dark blue) at a finite E -field by changing both spin and valley orientations, while retaining the orbital index. In the many-body picture, however, electrons favor a coherent superposition of the two states at the transition between the CAF and layer-polarized phases. The $\nu = \pm 2$ QHSs are present at $E = 0$, vanish at a small E -field, and then re-emerge (Fig. 3B and fig. S3). The $\nu = \pm 2$ at $E = 0$ can be explained as layer-coherent QHSs, where the LLs with the same N and spin orientation but different layer (valley) degrees of freedom form coherent superpositions (8).

Through the chemical potential mapping, we determine the LL energies in bilayer graphene as a function of ν and B -field. Figure 4A shows the bottom bilayer ρ_{xx} measured at $B = 12$ T, with the top bilayer charge neutrality line transposed on the contour plot (white line). Note that

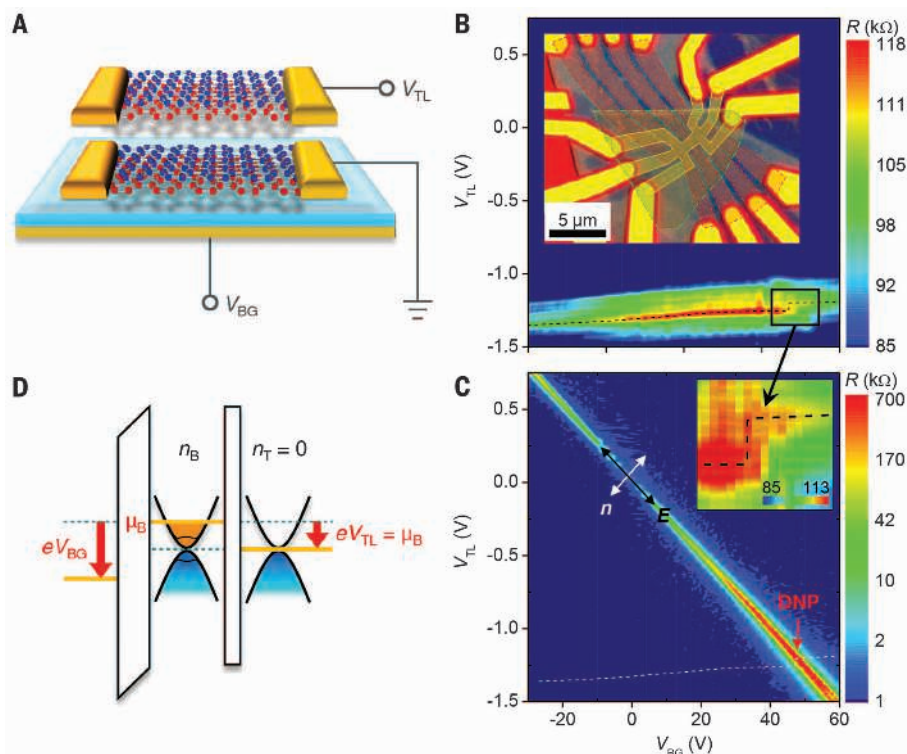


Fig. 1. Sample schematic and characterization. (A) Schematic of the double bilayer heterostructure. Top (B) and bottom (C) bilayer resistance R measured as a function of V_{TL} and V_{BG} . Dashed lines in the main plots of (B) and (C) mark the charge neutrality of the top bilayer. The bottom bilayer n and E -field axes are marked in (C). Inset in (B) shows a false-color optical micrograph of the device. The yellow (dashed red) contour marks the top (bottom) bilayer; the dashed green line marks the inter-bilayer hBN perimeter. Inset in (C) shows a magnified view of the top bilayer charge neutrality line near the double neutrality point (DNP). (D) Energy band profile across the heterostructure with the top layer at charge neutrality.

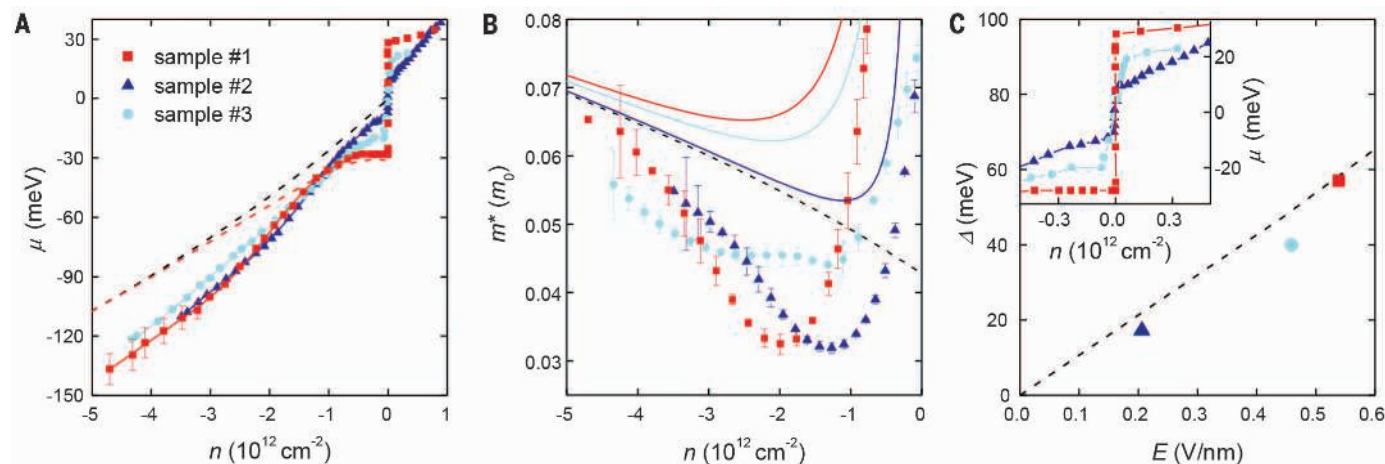


Fig. 2. Chemical potential and effective mass dependence on carrier density. (A) Bottom bilayer μ versus n in samples #1 to 3. Dashed lines: μ versus n calculated using the tight binding model for $E = 0$ (black) and $E = 0.54$ V/nm (red). (B) Symbols: measured m^* in units of bare electron mass (m_0) versus n . Also displayed are the m^* versus n calculated at $E = 0$ (black dashed), 0.54 V/nm (red), 0.21 V/nm (dark blue), and 0.46 V/nm (light blue). (C) Measured (symbols) and calculated (19) (dashed line) Δ values versus E -field. Inset shows a magnified view of the μ versus n near charge neutrality.

the transverse E -field across the bottom bilayer at DNP is 0.54 and 0.21 V/nm for sample #1 and #2, respectively, which leads to a $\nu = 0$ QHS in the bottom bilayer that is layer polarized at the DNP (supplementary text and fig. S4). At each integer filling of the bottom bilayer, the top bilayer neu-

trality line displays an abrupt change in the V_{TL} value, which translates into a chemical potential jump of bottom bilayer. The staircase-like dependence, particularly sharp at $\nu = 0, -4, -8, -12, -16$, testifies to a reduced LL broadening, in contrast to previous measurement in double monolayer

heterostructures using metal oxide as interlayer dielectric (15).

Figure 4B represents the LL orbital energies as a function of B -field and N , determined from the chemical potential at the half filling of each LL orbital index. The LL orbital energies increase

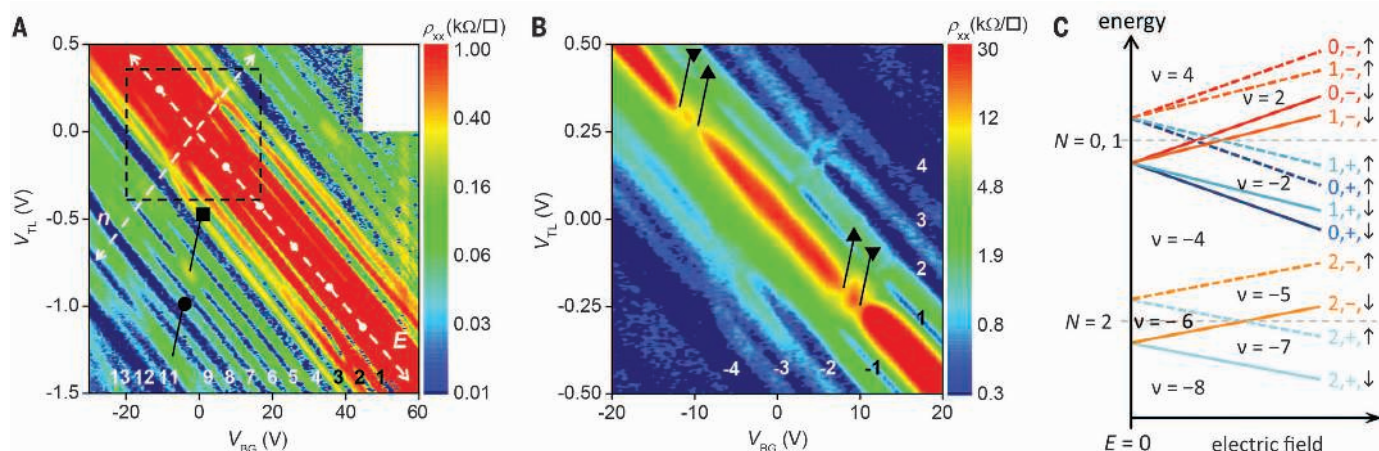
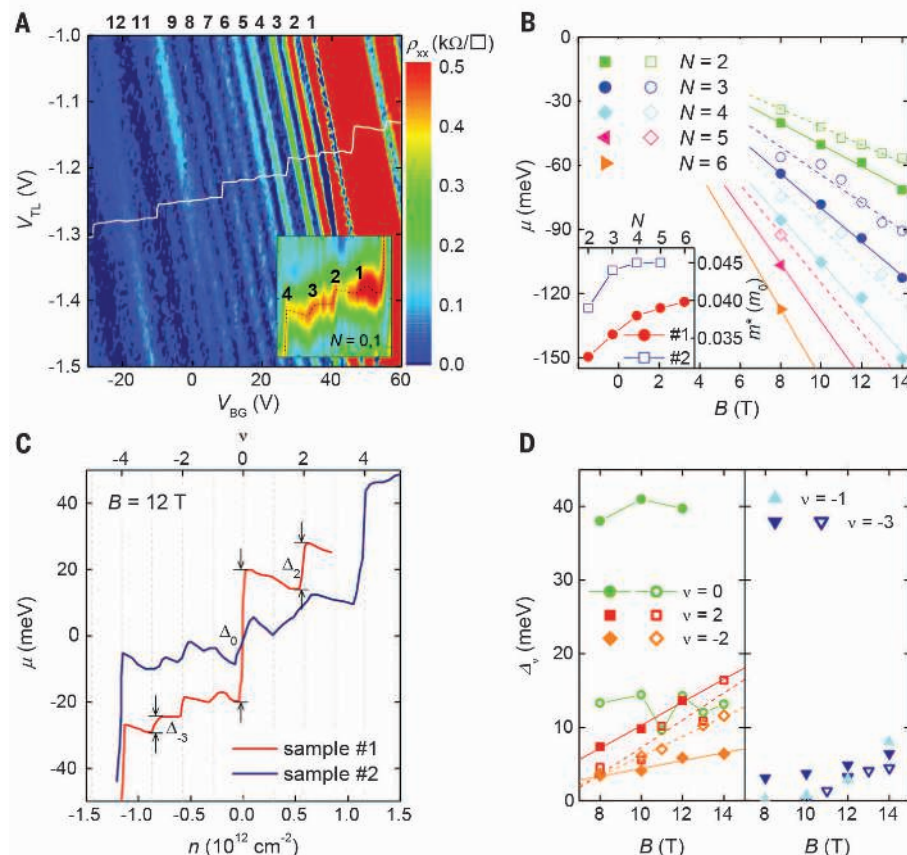


Fig. 3. Bilayer graphene quantum Hall ferromagnetism. (A) The bottom bilayer resistivity ρ_{xx} as a function of V_{TL} and V_{BG} , at $B = 14$ T and $T = 1.5$ K in sample #1. The dashed white lines mark the $n = 0$ and $E = 0$ axes; the dots along the $n = 0$ axis mark E -field increments of 0.1 V/nm. The absolute values of the QHS filling factors are shown. The E -field controlled spin-to-valley polarization transitions in the $N = 2$ (square) and $N = 3$ (circle) LLs are marked. (B) Magnified view of the dashed line rectangle of (A). The

triangles mark two distinct transitions of the $\nu = 0$ QHS as a function of E -field. (C) Schematic representation of the LL evolution with E -field, and the ensuing QHSs. The solid (dashed) line marks the spin down (up) levels. The orbital index and layer (+, -) degrees of freedoms are color coded. Assuming the direction of the applied E -field favors energetically the occupation of the bottom layer, the symbols +, - mark the bottom and top layers, respectively.

Fig. 4. Landau level energies and broken-symmetry gaps in bilayer graphene. (A) The bottom bilayer resistivity ρ_{xx} as a function of V_{TL} and V_{BG} at $B = 12$ T and $T = 1.5$ K in sample #1, along with the top bilayer charge neutrality locus (white line). The top axis shows the absolute values of the QHS filling factors. The scale bar corresponds to main panel data. Inset shows the top bilayer resistance dependence on V_{TL} and V_{BG} , as the top bilayer charge neutrality line crosses the $\nu = -4, -3, -2, -1, 0$ QHSs of the bottom bilayer; data range from 270 kilohm (blue) to 350 kilohm (red). (B) Orbital LL energies as a function of B -field and LL index in samples #1 (filled symbols) and #2 (open symbols). The inset shows the m^* versus N extracted from the main panel data. (C) Bottom bilayer μ versus n (bottom axis) and ν (top axis) at $B = 12$ T. (D) QHS gaps at $\nu = -3, -2, -1, 0, 2$ in sample #1 (filled symbols) and #2 (open symbols).



linearly with B -field, consistent with the theoretical $\mu = \hbar\omega_c\sqrt{N(N-1)}$ dependence (3); here $\omega_c = eB/m^*$ is the cyclotron frequency. The inset of Fig. 4B shows the m^* versus N dependence determined from the slope of μ versus B at each LL. The increasing m^* with N is similar to $B = 0$ T data of Fig. 2B.

The chemical potential of $N = 0, 1$ LLs, shown in Fig. 4A inset and Fig. 4C, reveals jumps at integer fillings $\nu = -3, -2, -1, 0$, and 2 , as well as a decreasing μ versus ν (or n) in the proximity of the QHSs stabilized in the $N = 0, 1$ LLs. The decreasing μ versus n dependence stems from a strong exchange interaction and translates into a negative compressibility of the electron system (23). A similar observation has been reported in monolayer graphene (24).

Using the chemical potential jump at integer fillings, we determine the broken-symmetry QHS gaps (Δ_0) at filling factors $\nu = -3, -2, -1, 0, 2$ as a function of B -field (Fig. 4D). We note that the measured Δ_0 is independent of B -field, in contrast to the linear B -field dependence of Δ_0 observed in single-gated bilayer graphene (II, 25). However, the data in Fig. 4D represent Δ_0 in the layer-polarized phase, whereas (II) and (25) probe Δ_0 in the CAF phase. The layer-polarized Δ_0 is controlled by the interaction and Zeeman splitting, as well as the E -field-induced on-site energy difference. At moderate E -fields, the competition between the single-particle splitting, which decreases with the B -field, and the $\propto\sqrt{B}$ interaction term leads to a Δ_0 weakly dependent on B -field and mainly controlled by the E -field (8). This explains the larger (smaller) Δ_0 value in sample #1 (#2), due to the larger (smaller) bottom layer E -field near the DNP.

The gaps of $\nu = -3, -1, -2$, and 2 have a linear dependence on the B -field, a trend similar to results obtained in single-gated suspended bilayer graphene (II). Whereas theoretical considerations (6, 8) suggest a sublinear B -field dependence associated with the $\propto\sqrt{B}$ of the interaction energy, the nonlinearity is weak, particularly in the B -field range probed here. Furthermore, the broken-symmetry QHSs observed in the $N = 0, 1$ LLs show a marked electron-hole asymmetry. Specifically, Δ_2 is larger than Δ_{-2} for both samples #1 and #2, whereas Δ_1 and Δ_3 are smaller than Δ_{-1} and Δ_{-3} , respectively; Δ_1 and Δ_3 are too small to be resolved experimentally. The electron-hole asymmetry and the differences in Δ_2 and Δ_{-2} in the two samples with different applied E -fields (Fig. 4D) observed experimentally are qualitatively consistent with a detailed Hartree-Fock analysis (8) of the broken-symmetry QHSs of the $N = 0, 1$ LLs.

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SUPPLEMENTARY MATERIALS

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BILAYER GRAPHENE

Tunable fractional quantum Hall phases in bilayer graphene

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Symmetry-breaking in a quantum system often leads to complex emergent behavior. In bilayer graphene (BLG), an electric field applied perpendicular to the basal plane breaks the inversion symmetry of the lattice, opening a band gap at the charge neutrality point. In a quantizing magnetic field, electron interactions can cause spontaneous symmetry-breaking within the spin and valley degrees of freedom, resulting in quantum Hall effect (QHE) states with complex order. Here, we report fractional QHE states in BLG that show phase transitions that can be tuned by a transverse electric field. This result provides a model platform with which to study the role of symmetry-breaking in emergent states with topological order.

The fractional quantum Hall effect (FQHE) (1) represents a notable example of emergent behavior in which strong Coulomb interactions drive the existence of a correlated many-body state. In conventional III-V heterostructures, the Laughlin wave function (2) together with the composite fermion picture (3) provides a complete description of the series of FQHE states that have been observed within the lowest Landau level (LL). In higher LLs, many-body states that are not amenable to this description appear, such as the even-denominator 5/2 state (4) [presumed to have non-abelian quantum statistics (5)] and a variety of charge-density wave states (6, 7).

In graphene (8–14), the combined spin and valley degrees of freedom are conjectured to yield FQHE states within an approximate SU(4) symmetry space (neglecting relatively weak spin Zeeman and short-range interaction energies). Although phase transitions in FQHE states have been explored for typical semiconductor systems (including valley degenerate systems) (15–20), the wide gate tunability of graphene systems coupled with large cyclotron energies allows for the exploration of multiple different SU(4) order parameters for a large range of filling fractions. In bilayer graphene (BLG), coupling to electric and magnetic fields can force transitions between different spin and valley orderings, including different FQHE phases (21–28). Thus, BLG provides a model system particularly well suited to experimentally study phase transitions between different topologically ordered states.

Whereas the FQHE in monolayer graphene has now been observed in several studies (8, 9, 11, 12), including evidence of magnetic-field-induced phase transitions (29), achieving the necessary sample quality in BLG has proven challenging (10, 13, 14). We fabricated BLG devices encapsulated in hexagonal boron nitride using the recently developed van der Waals transfer technique (30)

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[(31), section 1.1]. The device geometry includes both a local graphite bottom gate and an aligned metal top gate (Fig. 1A), which allows us to independently control the carrier density in the channel [$n = (C_{\text{TG}}V_{\text{TG}} + C_{\text{BG}}V_{\text{BG}})/e - n_0$, where C_{TG} is the top gate capacitance per area, C_{BG} is the bottom gate capacitance per area, V_{TG} is the top gate voltage, V_{BG} is the bottom gate voltage, e is the electron charge, and n_0 is residual doping] and the applied average electric displacement field [$D = (C_{\text{TG}}V_{\text{TG}} - C_{\text{BG}}V_{\text{BG}})/2\epsilon_0 - D_0$, where D_0 is a residual displacement field due to doping]. Crucially, portions of the graphene leads in these devices extend outside of the dual-gated channel. We used the silicon substrate as a third gate in order to set the carrier density of the leads independently (Fig. 1A). Tuning the carrier density in the graphene leads has a dramatic effect on the quality of magnetotransport data [(31), section 2.2], especially at large applied magnetic fields. Because of a slight systematic n-doping of our contacts during fabrication, our highest-quality data are obtained for an n-doped channel. We thus restricted our study to the electron side of the band structure.

Under application of low magnetic fields, transport measurements show a sequence of QHE plateaus in R_{xx} appearing at $h/4me^2$, where m is a nonzero integer and h is Planck's constant, together with resistance minima in R_{xx} , which is consistent with the single-particle LL spectrum expected for bilayer graphene (32). At fields larger than ~ 5 T, we observed complete symmetry breaking, with QHE states appearing at all integer filling fractions (fig. S8A), which is indicative of the high quality of our sample (Fig. 1B). By cooling the sample to sub-kelvin temperatures (20 to 300 mK) and applying higher magnetic fields (up to 31 T), clearly developed FQHE states appear at partial LL filling, with vanishing R_{xx} and unambiguous plateaus in R_{xx} (Fig. 1C). By changing V_{TG} while sweeping V_{BG} , we can observe the effect of different electric displacement fields on these FQHE states. In Fig. 1D, $V_{\text{TG}} = 0.2$, the $\nu = 2/3$ and $\nu = 5/3$ FQHE states are clearly visible as minima in σ_{xx} whereas for $V_{\text{TG}} = 1.2$ V, the $\nu = 2/3$ state is completely absent, the $5/3$ state appears weakened, and a new state at $\nu = 4/3$ becomes visible. This indicates that both the existence of the FQHE in BLG, and the sequence of the observed states, depend critically on the applied electric displacement field and that a complete study of the fractional hierarchy in this material requires the ability to independently vary the carrier density and displacement field.

To characterize the effect of displacement field in more detail, it is illuminating to remap the conductivity data versus displacement field and LL filling fraction ν (Fig. 2A). Replotted in this way, a distinct sequence of transitions, marked by compressible regions with increased conductivity, is observed for each LL. For example, at $\nu = 1$ there is evidently a phase transition exactly at $D = 0$ and then a second transition at large finite D . In contrast, at $\nu = 2$ (Fig. 2B) there is no apparent transition at $D = 0$, and a finite D transition appears at a much smaller displacement

field than at $\nu = 1$. Last, at $\nu = 3$ there is a single transition only observed at $D = 0$. This pattern is in agreement with other recent experiments (33). At large displacement fields, it is expected that it is energetically favorable to maximize layer polarization, indicating that low-displacement-field states that undergo a transition into another state at finite displacement field (such as $\nu = 1, 2$) likely exhibit an ordering different from full layer polarization. Following predictions that polarization in the 0-1 orbital degeneracy space is energetically unfavorable (34), this could be a spin ordering, like ferromagnetism or antiferromagnetism, or a layer-coherent phase (34–37). This interpretation is consistent with several previous experimental studies that reported transitions within the symmetry-broken integer QHE states to a layer-polarized phase under a finite displacement field (21–25, 33, 38).

At higher magnetic field and lower temperature (Fig. 2C), the integer states remain robust at all displacement fields, indicating that the observed transitions points in Fig. 2A exhibit insulating temperature dependence. This could be due to a small persistent bulk gap or disorder effects. At these fields and temperatures, FQHE states within each LL become evident, exhibiting transitions of their own with displacement field. Of particular interest are the states at $\nu = 2/3$ and $\nu = 5/3$, which are the most well developed. A strong $\nu = 2/3$ state is consistent with recent theory (39), which predicts this state to be fully polarized in orbital index in the 0 direction. At

$\nu = 2/3$, there is a transition at $D = 0$, as well as two more at $|D| \approx 100$ mV/nm (shown in Fig. 2C by the increased conductivity at the top and bottom boundaries of the plot. Behavior at larger $|D|$ is shown in fig. S8B). These transitions are qualitatively and quantitatively similar to the transitions in the $\nu = 1$ state seen in Fig. 2C. For the $5/3$ state, we present high-resolution scans at fields from 20 to 30 T in Fig. 2D. We again observed a transition at $D = 0$ as well as at finite D . The finite D transitions, however, occur at much smaller values than for either $\nu = 2/3$ or 1. Indeed, they are much closer in D value to the transitions taking place at $\nu = 2$, which are a factor of ~ 8 smaller than that of $\nu = 1$.

In Fig. 3, A and B, we plot the resistance minima of $2/3$ and $5/3$ state, respectively, as a function of D (corresponding to vertical line cuts through the two-dimensional map in Fig. 2C). For the $2/3$ state, we observed broad transitions, whose positions we define by estimating the middle points. Where the transition cannot be fully resolved because of limited gate range, this is done by the identification of a local minimum in conductance within the transition. The $5/3$ state exhibits much narrower transitions, which we mark by the local maximum of resistance. We observed some small asymmetry (<5 mV/nm) between the negative and positive D transitions, the source of which is not clear to us and which we include in our error associated with transition D values. The location of transitions in D is plotted in Fig. 3C as a function of magnetic field

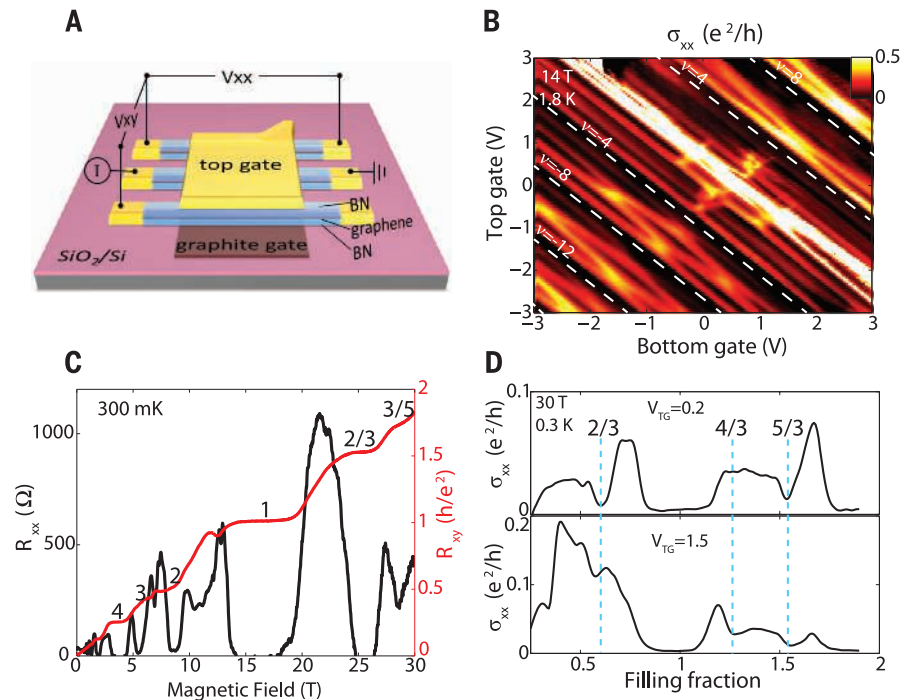


Fig. 1. Experimental setup and FQHE states. (A) Diagram of the dual-gate device architecture. (B) σ_{xx} as a function of top and bottom gate voltages at $B = 14$ T and $T = 1.8$ K. All broken-symmetry integer states are visible (multiple of 4 filling factors are marked with white dashed lines). (C) R_{xx} and R_{xy} as a function of magnetic field at a fixed carrier density ($n = 4.2 \times 10^{11} \text{ cm}^{-2}$). A fully developed $\nu = 2/3$ state appears at ~ 25 T, with a $3/5$ state developing at higher field. (D) σ_{xx} versus filling fraction at 30 T and 300 mK acquired by sweeping the bottom gate for two different top gate voltages.

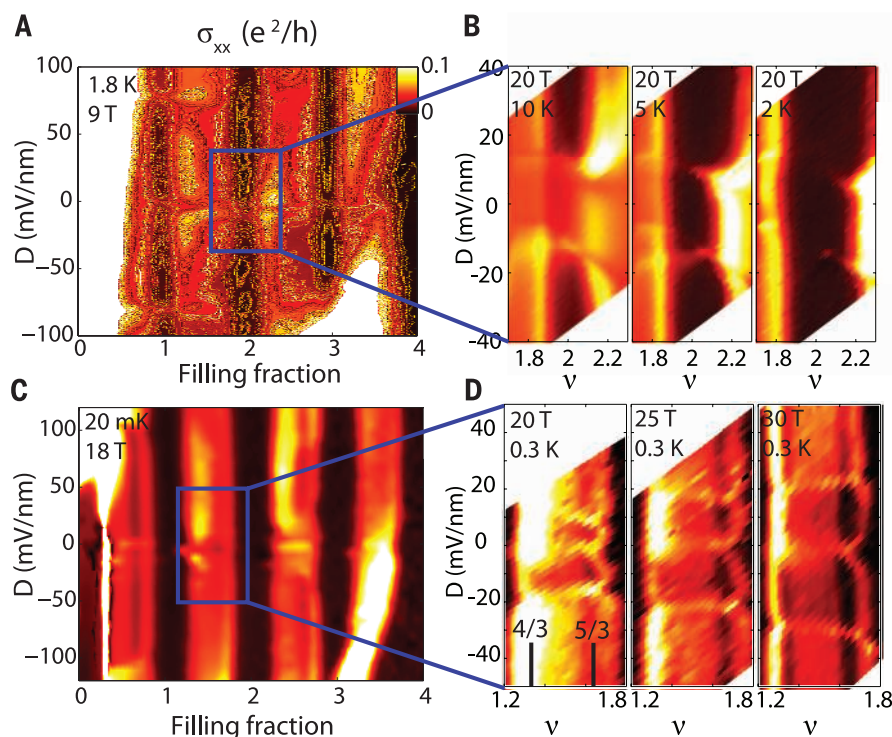


Fig. 2. Displacement field dependence. (A) σ_{xx} at $B = 9$ T and $T = 1.5$ K versus filling fraction and displacement field showing full integer symmetry breaking for $1 \leq \nu \leq 4$. Color scale applies to all plots in figure. (B) High-resolution scans of σ_{xx} for the $\nu = 2$ gap, $-40 < D < 40$ mV/nm at 20 T for three different temperatures. (C) σ_{xx} at $B = 18$ T and $T = 20$ mK showing both well-developed minima at fractional filling factors, and transitions with varying displacement field. (D) High-resolution scans of σ_{xx} for the region $1 < \nu < 2$, $-50 < D < 50$ mV/nm at 300 mK for three different magnetic fields.

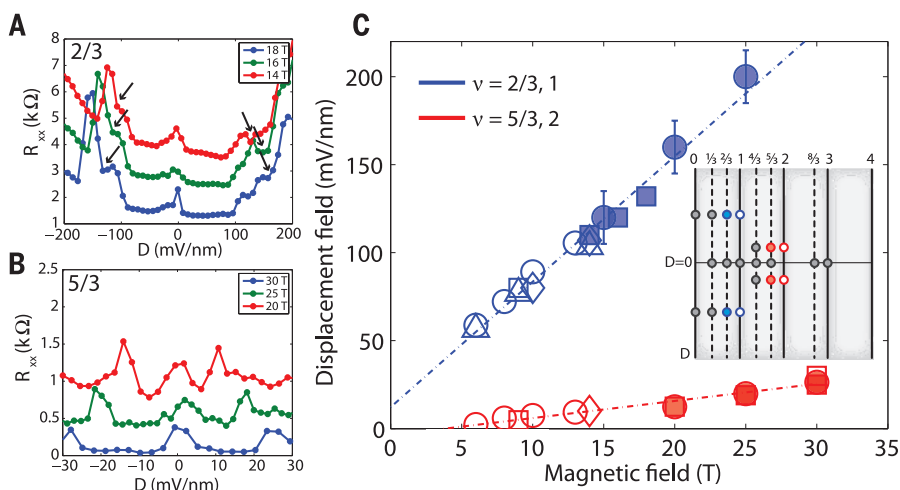


Fig. 3. Dependence of transitions on magnetic field. (A) R_{xx} versus displacement field at filling fraction $\nu = 2/3$ acquired at three different magnetic fields. Arrows mark transition middle points. Data are offset for clarity. (B) Similar data as in (A) but corresponding to $\nu = 5/3$. (C) All observed non- $D = 0$ transitions for $\nu = 2/3, 1$ (blue), $5/3$, and 2 (red). Open symbols correspond to integers, and solid symbols correspond to fractions. The data was acquired from four different devices, with each shape corresponding to a different device. Error bars are not shown where they are smaller than the markers. (Inset) A schematic map of the transitions in the symmetry broken QHE states for $0 \leq \nu \leq 4$. Vertical lines correspond to the QHE state for the filling fraction at the top. Boxes located on the central horizontal line indicate transitions at $D = 0$, and boxes located away from the horizontal line indicate transitions at finite D . Solid and open colored circles represent the transitions in (C), the main graph, whereas the black circles represent other transitions presented in Fig. 2.

B for both integer and fractional filling fractions. Our main observation is that the transitions in the fractional quantum Hall states and the transitions in the parent integer state (the smallest integer larger than the fraction) fall along the same line in D versus B . More specifically, the finite D transitions for $\nu = 2/3$ and $\nu = 1$ fall along a single line of slope 7 mV/nm $\cdot$ T, and the transitions for $\nu = 5/3$ and $\nu = 2$ fall along a single line of slope 0.9 mV/nm $\cdot$ T. This difference of a factor of ~ 8 in the slopes of the transition lines suggests a difference in the nature of the competing broken symmetry states at $\nu = 1$ and $\nu = 2$. The quantitative agreement observed between multiple devices indicates that these values are largely sample-independent.

We now turn to a discussion of the nature of these transitions. For the lowest LL of BLG, the possible internal quantum state of electrons comprises an octet described by the SU(4) spin-valley space and the 0-1 LL orbital degeneracy. Because $\nu = 1$ corresponds to filling five of the eight degenerate states in the lowest LL, the system will necessarily be polarized in some direction of the SU(4) spin-valley space. The presence of a $D = 0$ transition for $\nu = 1$ indicates that even at low displacement field, the ground state exhibits a layer polarization that changes as D goes through zero. At the same time, we expect that at large D the system is maximally layer polarized. We therefore propose that the transition at finite D is between a $1/5$ layer-polarized state (for example, 3 top layer and 2 bottom layer levels filled) and a $3/5$ layer-polarized state (for example, 4 top layer and 1 bottom layer levels filled) (35–37). The quantitative agreement of the transitions for $\nu = 2/3$ and $\nu = 1$, shown in Fig. 3C, strongly suggest that the composite fermions undergo a similar transition in layer polarization.

Whereas the observed transition at $\nu = 1$ and its associated FQHE ($\nu = 2/3$) can be explained by a partial-to-full layer polarization transition, the nature of the transition at $\nu = 2$ is less clear. The high D state presumably also exhibits layer polarization, but we do not have any experimental insight as to the ordering of the low D state. Additionally, although the finite D transitions in the $5/3$ state seem to follow the $\nu = 2$ transitions quantitatively (Fig. 3C), the $5/3$ state also has a clear transition at $D = 0$, suggesting that there may be a different ground state ordering of the $5/3$ and 2 states very near $D = 0$. In particular, the transition at $D = 0$ indicates that the $\nu = 5/3$ state exhibits layer polarization even at low displacement field, in a state separate from the high-displacement-field layer-polarized state, whereas this does not appear to be true at $\nu = 2$. One possible explanation for this result could be the formation of a layer-coherent phase that forms at $\nu = 2$ filling (37) but that is not stabilized at partial filling of the LL.

We also briefly mention other observed FQHE states. At the highest fields, we see evidence of weaker states at $\nu = 1/3$ and $\nu = 4/3$ (Fig. 2D) [(31), section 2.3], with both exhibiting phase transitions that appear to follow those of $\nu = 2/3$ and $\nu = 5/3$, respectively. We have also observed

transitions in the $\nu = 8/3$ state with displacement field (Fig. 2C), though we do not have a systematic study of its dependence on magnetic field because of our limited gate range. Qualitatively, there is a region at low D where no minimum is apparent that gives way to a plateau and minimum at finite D . The $\nu = 3$ state also exhibits a transition at $D = 0$ (Fig. 2A), indicating that there may also be a correspondence between the integer and fractional states in the $\nu = 3$ LL. These observations are summarized in Fig. 3C, inset; the detailed data are available in (37), section 2.6. Last, we have indications that the fractional hierarchy breaks electron-hole symmetry (14) [(37), section 2.3] because the clearest fractional states we observed can be described as $\nu = m - 1/3$, where m is an integer.

The electric-field-driven phase transitions observed in BLG's FQHE indicate that ordering in the SU(4) degeneracy space is critical to the stability of the FQHE. In particular, quantitative agreement between transitions in FQHE states and those in parent integer QHE states suggests that generally, the composite fermions in BLG inherit the SU(4) polarization of the integer state and couple to symmetry-breaking terms with the same strength. However, an apparent disagreement in the transition structure at $\nu = 5/3$ and $\nu = 2$ indicates that there may be subtle differences in the ground-state ordering for the integer and fractional quantum Hall states.

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Materials and Methods

Supplementary Text

Figs. S1 to S9

Reference (40)

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ACTIVE GALAXIES

A fast and long-lived outflow from the supermassive black hole in NGC 5548

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Supermassive black holes in the nuclei of active galaxies expel large amounts of matter through powerful winds of ionized gas. The archetypal active galaxy NGC 5548 has been studied for decades, and high-resolution x-ray and ultraviolet (UV) observations have previously shown a persistent ionized outflow. An observing campaign in 2013 with six space observatories shows the nucleus to be obscured by a long-lasting, clumpy stream of ionized gas not seen before. It blocks 90% of the soft x-ray emission and causes simultaneous deep, broad UV absorption troughs. The outflow velocities of this gas are up to five times faster than those in the persistent outflow, and, at a distance of only a few light days from the nucleus, it may likely originate from the accretion disk.

Outflows of photoionized gas are ubiquitous in active galactic nuclei (AGN) such as Seyfert galaxies (1, 2). Their impact on the environment can be estimated from the mass loss rate per solid angle, which is proportional to the hydrogen column density N_{H} , outflow velocity v , and distance r to the ionizing source. Whereas the first two quantities are directly obtained from high-resolution spectral observations, in most cases the distance can only be inferred from the ionization parameter $\xi = L/nr^2$, with n being the hydrogen density and L the ionizing luminosity between 13.6 eV and 13.6 keV. The density can be obtained from density-sensitive ultraviolet (UV) lines or by measuring the recombination time scale $t_{\text{rec}} \sim 1/n$ by monitoring the response of the outflow to continuum variations. The latter approach has recently been successfully applied to Mrk 509, providing strong constraints on the distances of the five ionization components in that source (3, 4).

Motivated by the successful results of the Mrk 509 campaign, we conducted comprehensive monitoring of NGC 5548, an archetypal Seyfert 1 galaxy ($z = 0.017175$). This campaign from May 2013 to February 2014 involved observations with the

X-ray Multi-Mirror Mission (XMM)–Newton, the Hubble Space Telescope (HST), Swift, the Nuclear Spectroscopic Telescope Array (NuSTAR), the International Gamma-Ray Astrophysics Laboratory (INTEGRAL), and Chandra satellites (5).

Unexpectedly, the soft x-ray flux in the first XMM-Newton observation (22 June 2013) was 25 times weaker than the typical median as measured with the Chandra Low Energy Transmission Grating Spectrometer (LETGS) in 2002, and this strong suppression was consistent throughout all 14 XMM-Newton spectra (Fig. 1). The NuSTAR and INTEGRAL spectra at energies above 10 keV show the characteristic power-law shape with a photon index of 1.6 to 1.7 and a weak constant reflection component, consistent with the median flux level of the 2002 Chandra LETGS observation. However, the spectrum is cut off below 10 keV, reaching an effective photon index of -0.5 in the 1- to 2-keV band before it flattens again below 1 keV. At energies <1 keV, the spectrum shows narrow emission lines and radiative recombination continua from photoionized gas at a temperature of ~ 6 eV. The intensity of the strongest emission line, the [O VII] forbidden line at 0.56 keV, is only slightly below its 2002 intensity.

These narrow features are superimposed on a weak continuum.

Comptonized spectra cannot explain the observed hard photon index. The central continuum is more likely absorbed by obscuring material that partially covers (~90%) the source. The absorbing gas must be inside the x-ray narrow line region; we concluded that this region is not obscured, because the intensity of the narrow emission features is not suppressed compared to archival data.

We obtained HST Cosmic Origins Spectrograph (COS) spectra concurrently with six of our 14 XMM-Newton spectra (Fig. 1). These UV spectra show a large number of narrow absorption lines at the velocities of the classical warm absorber (WA) components that were already present 20 years ago (1), but which now originate from less-ionized gas. This shows that the WA is now irradiated by a much lower ionizing flux than before.

After modeling the emission lines and continuum and accounting for the narrow absorption lines from the WA (5), broad, blue-shifted absorption troughs remain visible in the COS

spectra (Fig. 2). Asymmetric troughs reach their deepest point at outflow velocities of ~1000 km/s and extend to blueshifts as high as ~5000 km/s. All permitted UV transitions in the COS spectra show this blue-shifted absorption: C II, Si II, Si II*, S II, Fe III, C III*, Si III, Si IV, C IV, N V, and Ly α . As expected, no absorption is seen associated with forbidden or semiforbidden transitions or highly excited states such as He II λ 1640. As we show below, all visible broad absorption transitions appear in ions formed at ionization parameters similar to those needed to explain the x-ray obscuration. In addition, there is a hint of a correlation between the strengths of the broad absorption troughs in the individual observations and the strength of the x-ray obscuration (Fig. 2C), which may suggest that the broad UV absorption troughs and the x-ray obscuration arise in the same photoionized gas.

As indicated above, the UV manifestation of the persistent classical WA has a substantially lower degree of ionization because of this obscuration. Lower ionization will also cause a significant increase in the x-ray opacity of the WA that needs to be taken into account when modeling the obscuring medium in the x-ray band. Therefore, we developed a proper model for the 2013 WA (5) based on the physical characteristics of the WA and spectral energy density (SED) as measured in 2002 (6), when there was no obscurer present.

The best-fit model with a single obscuring component produced a very hard continuum spectrum with a photon index of 1.48, inconsistent with the high-energy NuSTAR data that are not affected by obscuration. We required two obscuring components to find a solution consistent with the hard x-ray spectrum (fig. S3), which has a photon index of 1.57.

The first obscuring component covers 86% of the x-ray source and has a hydrogen column density of $1.2 \times 10^{26} \text{ m}^{-2}$ and $\log \xi = -1.2$ (in units of 10^{-9} W m). This component, derived from x-ray analysis only, reproduces all the broad absorption troughs seen in the UV. The similar depths of red and blue transitions for Si IV, C IV, and N V in Fig. 2A indicate that the troughs are saturated. They only partially cover the broad lines and continuum, such that the line profiles indicate the velocity-dependent covering factor rather than the column density profile.

The troughs in C IV and Ly α are deep enough to fully cover the continuum emission, but it is not possible to unambiguously determine the separate broad-line and continuum covering fractions. If the continuum is fully covered, then the covering factor of the C IV broad line region (BLR) is 20%. For N V and Si IV, the maximum continuum covering fractions are 95% and 40%, respectively. For continuum covering fractions of 30% in each line, the BLR covering fractions of C IV, N V, and Si IV are 30%, 40%, and 20%, respectively. The signal in the Reflecting Grating Spectrometer (RGS) soft x-ray band is too low and dominated by features from the narrow emission lines and WA to allow useful constraints on outflow velocity or line width of the obscurer.

Therefore, the COS UV spectra are essential for understanding the dynamics of the outflow.

The second obscuring component covers 30% of the x-ray source with $N_{\text{H}} = 10^{27} \text{ m}^{-2}$ and is almost neutral. Taking the same ratio between the x-ray and the UV continuum covering factors as for component 1 (86% for x-ray, 30% for UV), the UV covering factor of component 2 is small (less than 10%).

The obscuration is present during our full campaign (Fig. 3). Archival Swift data (fig. S4) show large hardness ratios, a strong signature of photoelectric absorption, indicating the obscuration started between August 2007 and February 2012, when Swift was not monitoring NGC 5548; a HST/COS spectrum taken in June 2011 already indicates an onset of the broad absorption lines. Thus, the obscuration has lasted for at least 2.5 years and perhaps as long as 6 years.

In early September 2013, NGC 5548 brightened for about 2 weeks (Fig. 3). The Swift x-ray hardness ratio indicated that the source was still obscured, so the continuum itself must have increased. The last Chandra LETGS spectrum of 11 September 2013 showed the effect of the outburst as an increase in the ionization degree of the WA and reappearance of several x-ray lines from the WA but no noticeable effect on the obscurer.

What is the obscurer, and where is it located (Fig. 4)? The UV troughs are deeper than the continuum, and the obscurer partially covers the broad UV emission lines, implying a distance of more than 2 to 7 light days (10^{14} m) from the core (7). However, it strongly affects the WA by reducing the level of ionization, implying that the obscurer is within $\sim 10^{17} \text{ m}$ from the core (8). Given the UV broad line covering factor of obscurer component 1 of 20 to 40%, it is likely that the obscurer is close to the broad UV line-emitting region. On the basis of the UV/x-ray ratio of continuum covering factors determined before for the obscuring component 1, it follows that the UV source is only two times larger than the x-ray source. Another indication for a distance just outside of the BLR is the high velocity of the gas in the line of sight, peaking at ~1000 km/s and extending out to ~5000 km/s. We see variations in the obscuration between subsequent XMM-Newton observations, spaced by two days. Assuming that the x-ray source has a typical size of $10 R_g$ ($R_g = GM/c^2$, where G is the Newtonian gravitational constant) and a black hole mass $M = 3.9 \times 10^7$ solar masses (9), the velocity needed to cross this distance in 2 days is 3000 km/s, of the same order of magnitude as the velocities observed along the line of sight. On the other hand, this obscuration lasts for several years, indicating ongoing replenishment of the obscuring material, possibly from the accretion disk or from the BLR.

Given the location, the outflow velocity, and the changes in the x-ray covering fraction of the obscurer, we may attribute the obscuring material to a wind from the accretion disk reaching beyond the BLR. The line of sight is inclined by $\sim 30^\circ$ to the rotation axis of the disk (9), and these velocity components therefore

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indicate a predominantly poloidal outflow. This is more readily explained by magnetically confined acceleration than by radiative acceleration, which would lead to a more radial or equatorial outflow.

Evidence is accumulating for strong variations in the x-ray absorption properties along the line of sight for both type-1 and type-2 AGNs. X-ray absorbers show changes from Compton-thin to Compton-thick columns, changes in covering frac-

tion, eclipse or occultation events, variations in the ionization state, and velocity of some of their major components (10–18). Observations so far suggest that these examples of circumnuclear absorbing gas are inhomogeneous and clumpy,

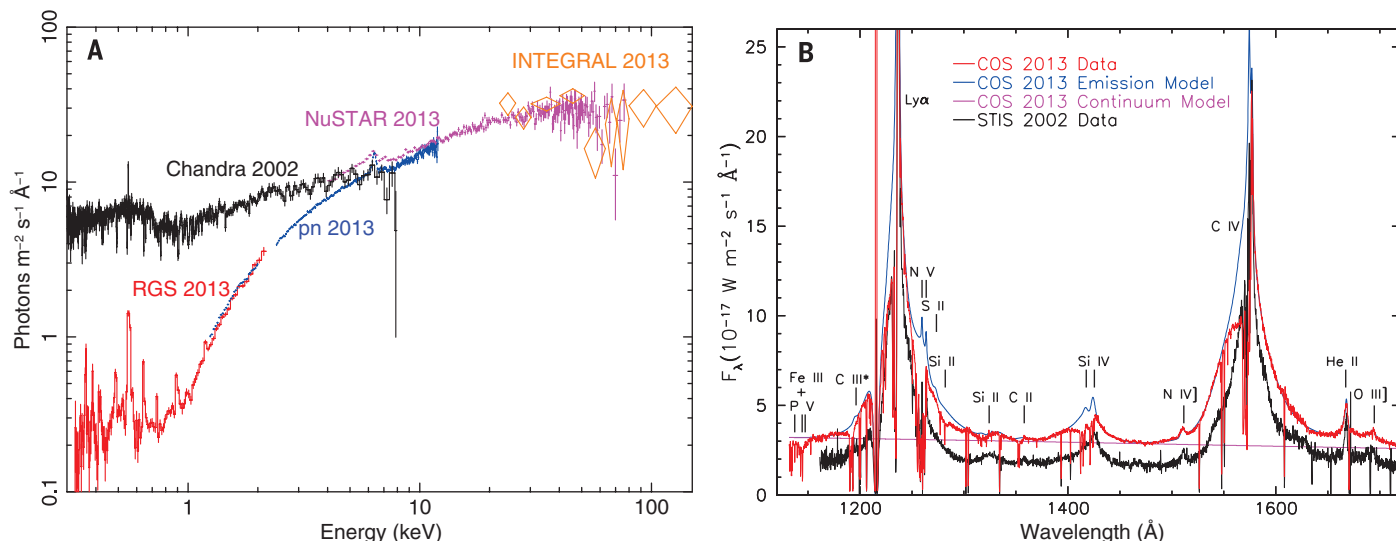


Fig. 1. X-ray and UV spectra of NGC 5548. All data have been rebinned for clarity. Error bars are ± 1 SD. **(A)** The heavily obscured x-ray spectrum during summer 2013. The unobscured Chandra LETGS spectrum taken in 2002 is shown for comparison. The 2013 spectrum is obtained from 12 XMM-Newton [European Photon Imaging Camera–pn detector (pn) and RGS], two NuSTAR, and four INTEGRAL observations; these latter two data sets were taken when the hard x-ray flux was 10% higher than the average >10 -keV flux of the XMM-Newton data. **(B)** Averaged 2013 COS spectrum F_λ as a function of wavelength compared with the 2002 STIS spectrum, showing the broad UV absorption lines in 2013.

Fig. 2. UV absorption lines in the COS spectrum of NGC 5548.

(A) The combined normalized spectra from summer 2013 have been binned for clarity. For the Si IV, C IV, and N V doublets, the red and blue profiles are registered relative to the respective red and blue wavelengths of the doublets. The smooth solid lines are fits to the BAL profile with 1:1 ratio for the doublets. The dotted vertical lines show the locations of the narrow velocity components defined in (8), with an additional component 6 near 0 velocity. **(B)** Time variability of the equivalent width (EW) of the C IV and Si IV broad absorption troughs (including the 2014 measurement). Error bars are ± 1 SD. **(C)** EWs of the C IV and Si IV broad absorption troughs, showing an anticorrelation with the Swift soft x-ray flux (0.3 to 1.5 keV). Error bars are ± 1 SD.

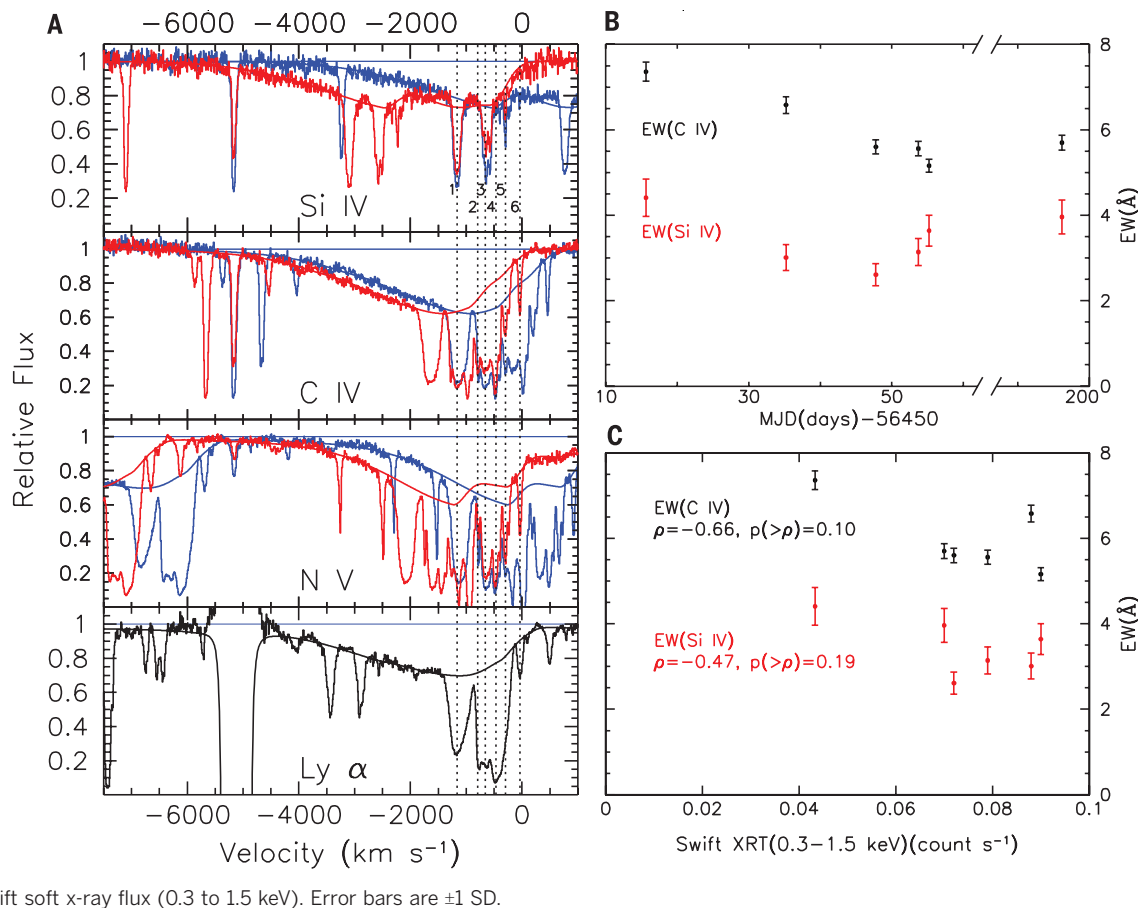


Fig. 3. X-ray and UV light curves of NGC 5548 obtained with Swift.

Horizontal dashed lines show the average flux or hardness measured with Swift during 2005 and 2007 in an unobscured state. The first and second shaded areas indicate the epochs of the first 12 XMM-Newton and the three Chandra observations, respectively. The outburst around day 80 was also obscured, as evidenced by the hardness ratio. Note the apparent anticorrelation between hardness and UV flux. Error bars are ± 1 SD; XRT, x-ray telescope; UVOT, ultraviolet/optical telescope; MJD, modified Julian date. The full light curve is shown in fig. S4.

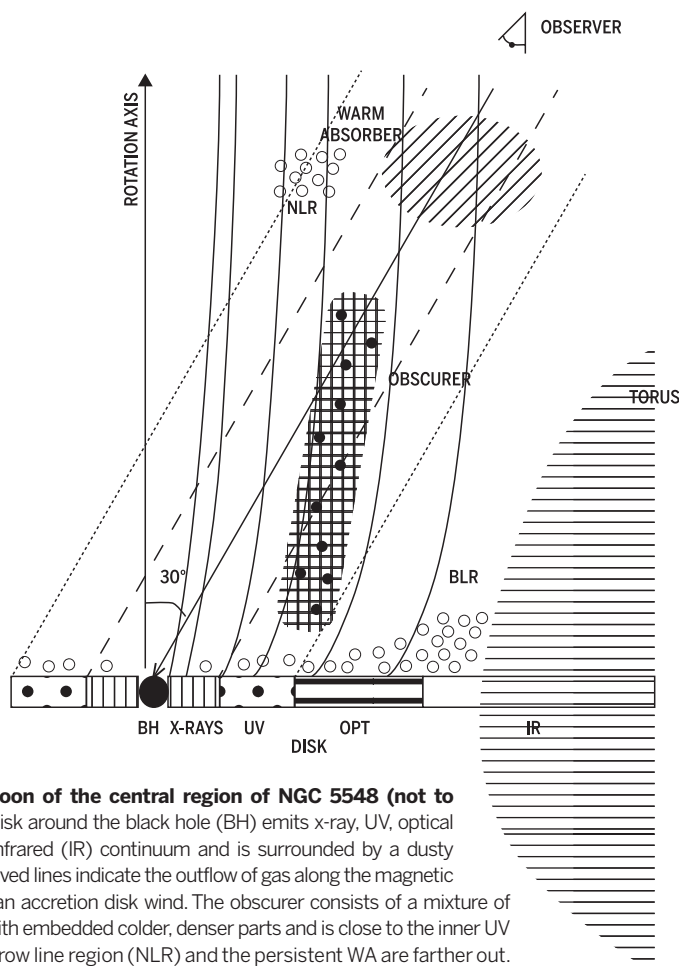
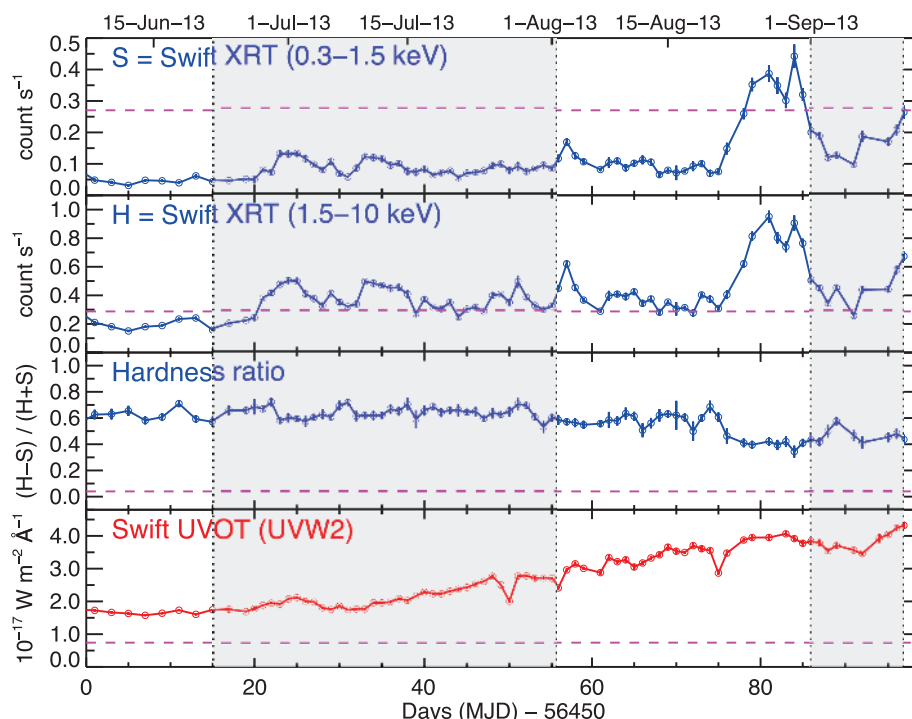


Fig. 4. Cartoon of the central region of NGC 5548 (not to scale). The disk around the black hole (BH) emits x-ray, UV, optical (OPT), and infrared (IR) continuum and is surrounded by a dusty torus. The curved lines indicate the outflow of gas along the magnetic field lines of an accretion disk wind. The obscurer consists of a mixture of ionized gas with embedded colder, denser parts and is close to the inner UV BLR. The narrow line region (NLR) and the persistent WA are farther out.

preferentially distributed along the equatorial plane, and possibly resulting from either a clumpy torus or clouds of the BLR passing along the line of sight. On the other hand, the distant WA seems to persist over much longer time scales.

Compared with previous studies, our observations of this type-1 AGN stand out because (i) this persistent x-ray/UV-obscuring event implies a long, inhomogeneous stream of matter, possibly the onset of an accretion disk wind, and (ii) this phenomenon was detected simultaneously in x-rays and UV.

Outflows powerful enough to influence their environments are present in luminous quasars like broad-absorption line (BAL) quasi-stellar objects (QSOs). Theoretical models of radiatively driven accretion-disk winds in BAL QSOs require that the accelerated UV-absorbing gas is shielded from much of the UV/x-ray ionizing radiation (19). They predict heavy x-ray obscuration and broad UV absorption lines arising from gas near the accretion disk. Observational evidence is ambiguous, because S. Gallagher *et al.* (20) see evidence for both x-ray absorption and intrinsically weak x-ray emission in BAL QSOs.

The proximity and brightness of nearby Seyfert galaxies make them ideal laboratories for studying the mechanisms that drive the powerful winds seen in their more-luminous cousins.

Our observations of NGC 5548 show that the x-ray obscuration and the fast broad UV outflow both arise in the region expected for an accretion disk wind, and we established that this shielding gas is near the BLR.

Although the outflow in NGC 5548 is not strong enough to influence its host galaxy, it gives us unique insight into how the same mechanism may be at work in much more powerful quasars.

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SUPPLEMENTARY MATERIALS

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C–H BOND ACTIVATION

Regioselective ketone α -alkylation with simple olefins via dual activation

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Alkylation of carbonyl compounds is a commonly used carbon-carbon bond-forming reaction. However, the conventional enolate alkylation approach remains problematic due to lack of regioselectivity, risk of overalkylation, and the need for strongly basic conditions and expensive alkyl halide reagents. Here, we describe development of a ketone-alkylation strategy using simple olefins as the alkylating agents. This strategy employs a bifunctional catalyst comprising a secondary amine and a low-valent rhodium complex capable of activating ketones and olefins simultaneously. Both cyclic and acyclic ketones can be mono- α -alkylated with simple terminal olefins, such as ethylene, propylene, 1-hexene, and styrene, selectively at the less hindered site; a large number of functional groups are tolerated. The pH/redox neutral and byproduct-free nature of this dual-activation approach shows promise for large-scale syntheses.

The α -alkylation of carbonyl compounds, an old but fundamental organic transformation, is still widely used in complex molecule syntheses (1). Conventionally, carbonyl alkylation involves generation of metal enolates followed by addition of an alkylating agent, often alkyl halides (Fig. 1A). Although effective, as documented in almost all organic chemistry textbooks, this enolate alkylation approach suffers from many drawbacks (1), including (i) the need for stoichiometric strong metallic bases (e.g., lithium diisopropylamide) and cryogenic conditions (to avoid homolytic couplings); (ii) the challenge in controlling regioselectivity for unsymmetrical ketones and curtailing overalkylation to di- or trisubstituted products; (iii) the expense of alkyl halide reagents (2–4); and (iv) the formation of stoichiometric metal halides and conjugate acids of the bases as byproducts. On the other

hand, the Stork enamine reaction (5, 6) (Fig. 1B) affords monoalkylation with high regioselectivity at the less hindered α carbons under less basic conditions; however, use of reactive alkylating agents (alkyl halides or Michael acceptors) is still required due to the reduced nucleophilicity of enamines versus metal enolates.

We foresaw substantial advantages in the prospective use of simple unactivated olefins as alkylating agents (Fig. 1C). Adding the ketone α -C–H bond across a C–C double bond under neutral conditions would furnish no byproducts and tolerate a broad range of functionality. This approach would also have economic advantages because olefins are much cheaper and more readily available feedstock than the corresponding alkyl halides (Fig. 1D); in fact, most terminal alkyl halides are ultimately prepared from olefins (4, 7).

Although adding α -C–H bonds of activated methylene compounds across olefins and alkynes has been established (8–11), there are fewer examples of direct coupling of simple ketones and unactivated olefins. The intramolecular ketone-olefin

coupling, known as the Conia-ene reaction (12), requires high temperature (>250°C), giving moderate yields with limited functional-group tolerance; the catalytic version was first reported by Widenhoefer (13) using palladium and recently by Che (14) using gold. In contrast, intermolecular ketone-olefin couplings are rare and mainly involve addition of stoichiometric metal enolates or enamides across olefins (8, 15, 16). To our knowledge, base-catalyzed additions of metal enolates to styrene derivatives (likely facilitated by formation of delocalized charges) (17, 18) and a Mn/Co-initiated oxidative radical process for nonaromatic olefins (19) are the only approaches reported to date. Recently, enamine radical cation-mediated couplings with olefins emerged as an attractive catalytic strategy for α -functionalization of carbonyl compounds, albeit requiring oxidative conditions (20, 21). Therefore, a general activation mode for coupling of simple ketones and unactivated olefins remains to be developed. Here, we describe our development of a catalytic dual-activation strategy for addition of normal ketone α -C–H bonds across unactivated olefins, which allows for direct ketone alkylation by simple olefins under both pH- and redox-neutral conditions.

We targeted a bifunctional catalyst capable of activating the ketone α -C–H bonds and the olefin simultaneously, which would incorporate a secondary amine and a low-valent transition metal complex. Seminal work by Jun and co-workers showed that metal-organic cooperative catalysis enables activation of aldehyde *ipso*-C–H bonds through imine formation with a bifunctional primary amine, i.e., 2-amino-3-picoline (22). We hypothesized that, by using a secondary amine, the ketone α -C–H bonds would instead be activated by enamine formation. As depicted in Fig. 2, first, the catalyst would bind the ketone substrate to form an enamine (step a), which would consequently convert the ketone α sp^3 C–H bond into a sp^2 C–H bond, thus enhancing the reactivity toward oxidative addition by a low-valent transition metal (23, 24). Meanwhile, if a proper directing group (DG) were linked to the amine domain, the DG could facilitate insertion of a low-valent transition metal (e.g., Rh^I) into the resulting enamine C–H bonds, giving

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metal hydride species (M–H, step b). Upon olefin coordination to the metal, subsequent M–H migratory insertion (step c) and reductive elimination (step d) would provide an alkylated enamine, which upon hydrolysis would lead to α -alkylation and catalyst regeneration (step e).

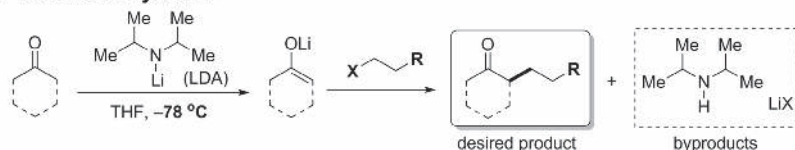
In the Stork enamine reaction (5, 6), enamine formation is regioselective for the less-hindered

site of ketones; thus, this bifunctional catalyst would be expected to provide high regiocontrol for monoalkylation of unsymmetrical ketones. Moreover, as documented in enamine catalysis (25), enamine formation and hydrolysis can exist in equilibrium, but the less-hindered ketone (starting material) preferentially forms enamine over the more-hindered ketone (product), and thus pro-

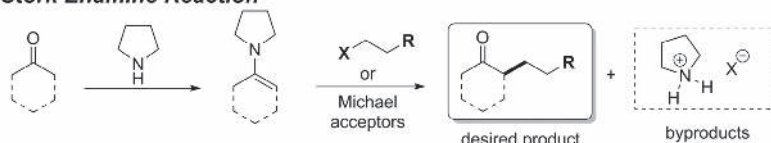
duct inhibition can be avoided. In addition, enamine formation/hydrolysis is known to be compatible with the Rh^I to Rh^{III} catalytic cycle (26). Therefore, both the amine and the metal components can be employed catalytically. [For our preliminary study of alkylation with 1,2-cyclic diketones using stoichiometric aminopyridine as the cofactor, see (27).]

To examine the feasibility of the proposed strategy, we tested 3-phenylcyclopentanone (**1a**) and ethylene (**2a**) as model substrates. A variety of rhodium precatalysts/dative ligands, bifunctional ligands, solvents, additives, and pressure of ethylene were examined (see table S1). Given the crucial role of the bifunctional ligands in the proposed catalytic cycle, seven secondary amine compounds (**L1** to **L7** in table S1) containing an adjacent DG were designed and explored under the ethylation conditions. To our delight, 7-azaindoline (**L1**) exhibited unique and high catalytic activities, whereas others were inactive. In the presence of 2.5 mole percent (mol %) chlorobis(cyclooctene) rhodium(I) dimer {[Rh(coe)₂Cl]₂}, 5 mol % 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene (IMes) ligand, 10 mol % *p*-toluenesulfonic acid monohydrate (TsOH·H₂O), and 25 mol % **L1** in toluene, the desired ethyl-substituted product **3a** was isolated in 82% yield using ethylene gas as the alkylating agent (table S1, entry 4). Subsequently, the role of each reactant was investigated through a series of control experiments. The absence of the Rh^I complex or bifunctional ligand **L1** completely eliminates the reactivity (table S1, entries 5 and 6), supporting our hypothesis that enamine formation and low-valent metal are key for the C–H/olefin coupling reaction. To promote enamine formation, 10 mol % TsOH·H₂O was purposely employed as an acid catalyst; indeed, without this additive, no desired alkylation product was observed (table S1, entry 7). The bulky electron-rich N-heterocyclic carbene (NHC) ligand (IMes) was used to promote oxidative addition of enamine C–H bonds to Rh and subsequent olefin insertion (28). Considerably diminished yields were found in the absence of this ligand or using Wilkinson's catalyst instead (table S1, entries 8 and 20). The reaction exhibited complete regioselectivity for the less-hindered 5 position of cyclopentanone; neither alkylation at the 2 position nor multiple alkylations was observed for ketone **1a**. Moreover, while the reaction requires 2 days at toluene reflux temperature when 2.5 mol % [Rh(coe)₂Cl]₂, 5 mol % IMes, and 25 mol % **L1** were employed, increasing the catalyst loading can result in a full conversion within 12 to 24 hours (table S1, entries 1 and 2). With the optimized conditions in hand, we examined the substrate scope. A wide range of different functional groups were tolerated under the alkylation conditions (Fig. 3A). Ethers, aryl bromides, carboxylic esters, methylenedioxy, nitriles, and thioethers proved compatible (**3b** to **3h**). Substrates containing competitive alkylation sites, such as secondary amides (**3i**), malonates (**3j**) and aliphatic esters (**3k** and **3t**), gave chemo- and regioselective ethylation exclusively at the ketone C5 position. Furthermore, reactive functional

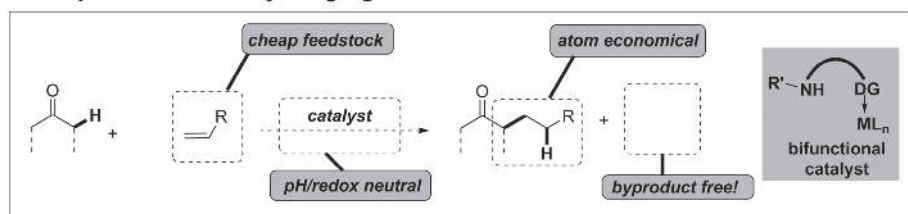
A Enolate Alkylation



B Stork Enamine Reaction



C Simple Olefins as Alkylating Agents



D Cost of Alkylating Agents

	H ₂ C=CH ₂	VS	ICH ₂ CH ₃	BrCH ₂ CH ₃
estimated cost:	\$1/kg (ICIS market price)		\$280/kg (Aldrich)	\$55/kg (Aldrich)
	\$0.028/mol		\$43.7/mol	\$6.0/mol
molecular weight:	28		156	109

Fig. 1. Different approaches to ketone alkylation. (A) Enolate alkylation. (B) Stock enamine reaction. (C) Simple olefins as alkylating agents. (D) Cost of alkylating agents.

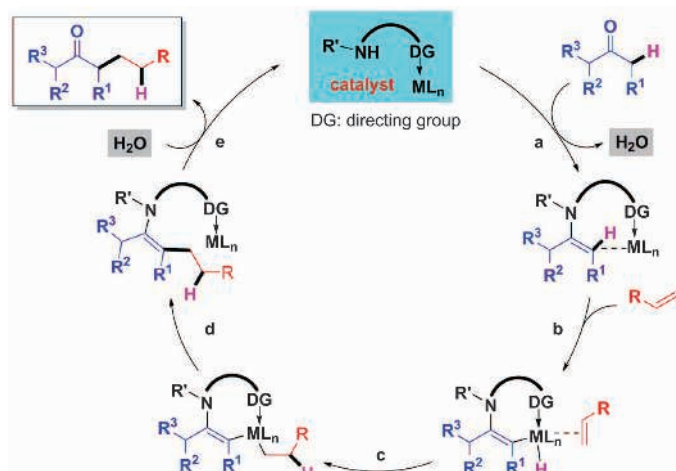


Fig. 2. Design of a bifunctional catalyst and proposed catalytic cycle. (a) Enamine formation; (b) Oxidative addition of enamine C–H bond; (c) Migratory insertion into olefins; (d) Reductive elimination to form C–C bond; (e) Enamine hydrolysis.

groups, including free tertiary and primary alcohols (**3l** and **3m**), free phenols (**3n**), unprotected indoles (**3o**), and amines (**3p**), survived, giving moderate to high yields of ketone-alkylation products. Acid-sensitive substrates, such as those containing a *tert*-butyldimethyl silyl (TBS) ether (**3q**), a tertiary alcohol (**3l**), and a trimethylsilyl

(TMS) group (**3ad** in Fig. 3C), were also suitable for this transformation. Furthermore, enolizable preexisting stereocenters were preserved during the reaction (**3t**) because no strong base was involved. Thus, this method provides complementary compatibility to the conventional enolate alkylation chemistry.

On a 2-mmol scale (2.0 M), the reaction of ketone **1a** provided full conversion and 96% isolated yield with a lower catalyst loading (versus the 0.2-mmol scale). In addition, using 10 mmol (0.98 g) 3-methylcyclopentanone as the starting material, we obtained the desired ethylation product (**3s**) in nearly quantitative yield [determined by ^1H nuclear

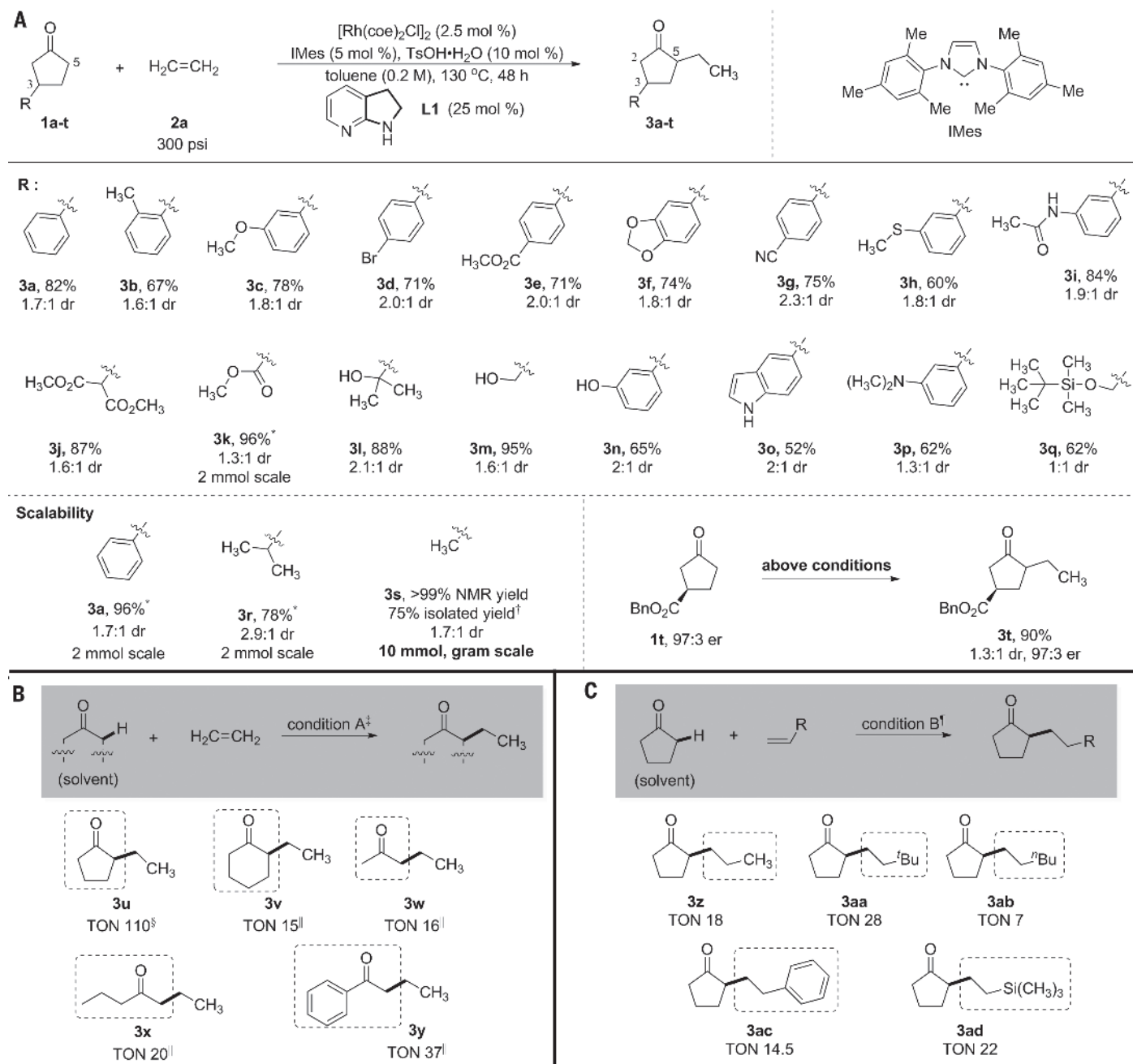


Fig. 3. Regioselective ketone α -alkylation with simple olefins. (A) Ethylation of various cyclopentanones. Unless otherwise mentioned, the reactions were run on a 0.2-mmol scale; the data are reported as percent isolated yield. Diastereomeric ratio (dr) was determined by ^1H NMR. * $[\text{Rh}(\text{coe})_2\text{Cl}]_2$ (1 mol %), IMes (2 mol %), $\text{TsOH}\cdot\text{H}_2\text{O}$ (5 mol %), toluene 1 mL (2.0 M). † $[\text{Rh}(\text{coe})_2\text{Cl}]_2$ (0.5 mol %), IMes (1 mol %), $\text{TsOH}\cdot\text{H}_2\text{O}$ (2 mol %), **L1** (15 mol %), toluene 3.0 mL (3.3 M). **(B)** Ethylation of various ketones. ‡Condition A: ketone 1 mL, ethylene 300 pounds per square inch, **L1** (0.2 mmol), $[\text{Rh}(\text{coe})_2\text{Cl}]_2$ (0.005 mmol), IMes (0.01 mmol), $\text{TsOH}\cdot\text{H}_2\text{O}$ (0.02 mmol), 2,4,4-trimethylpentan-2-amine

(0.1 mmol), neat, 130°C, 48 hours. The TON are based on [Rh] monomer, which are determined by GC with dodecane as an internal standard. §No 2,4,4-trimethylpentan-2-amine, but with H_2O (10 μL); 2,5-diethyl-cyclopentanones were also obtained (TON 29). ||180°C. **(C)** Alkylation of cyclopentanone with various olefins. ¶Condition B: cyclopentanone (0.5 mL), olefin (0.5 mL), propene ~1 mL, **L1** (0.2 mmol), $[\text{Rh}(\text{coe})_2\text{Cl}]_2$ (0.005 mmol), IMes (0.01 mmol), $\text{TsOH}\cdot\text{H}_2\text{O}$ (0.02 mmol), 2,4,4-trimethylpentan-2-amine (0.1 mmol), H_2O (10 μL), neat, 130°C, 48 hours. The TON are based on [Rh] monomer, which are determined by GC with dodecane as an internal standard.

magnetic resonance (NMR)] with only 0.5 mol % of the Rh-dimer catalyst and 15 mol % of L1 (75% isolated yield due to the volatility of **3s**). This reaction can tolerate a range of concentrations (from 0.2 M to 3.3 M), which can be critical for large-scale applications. Structures of the products were unambiguously characterized by $^1\text{H}/^{13}\text{C}$ NMR, infrared (IR), and high-resolution mass spectrometry (HRMS); x-ray structures of several hydrazone derivatives were also obtained. For all substrates, the products were obtained as a pair of diastereoisomers with the cis-isomer predominating. It is likely that in the last step (enamine hydrolysis), the proton preferentially attacks the less-hindered face of the enamine, resulting in cis disposition of the two substituents. The diastereomeric ratio (dr) of the alkylation product can be enhanced by conversion to the corresponding silyl enol ether followed by treatment with a chiral Lewis acid (supplementary materials).

We next explored the scope of ketones and olefins for this transformation. Both cyclic and acyclic ketones could be directly coupled with ethylene gas to afford the ethyl-substituted ketones (Fig. 3B). In general, cyclopentanones were more reactive than cyclohexanones and acyclic ketones, consistent with the established tenden-

cies toward enamine formation (29). By further investigating the reaction conditions, we discovered that a catalytic amount of an additional amine, such as triethylamine, 1,4-diazabicyclo[2.2.2]octane (DABCO), or 2,4,4-trimethylpentan-2-amine, could increase the efficiency of the ketone α -alkylation. Although the exact reason remains unclear, with the help of the amine additive, simple aliphatic ketones, such as acetone and 2-pentanone, coupled with ethylene to afford the desired monoalkylation products. It is well established that aromatic ketones, such as acetophenone, can undergo metal-catalyzed C-H/olefin couplings through activation of the *ortho* aromatic C-H bond, initially reported by Murai and co-workers (30). In contrast, our strategy completely switched the chemoselectivity from the normal aromatic C-H bond to the ketone α -C-H bond, providing homologated ketone **3y**.

Using cyclopentanone as the ketone substrate, different classes of terminal olefins were also explored. All these α -olefins provided the desired monoalkylation products with complete regioselectivity for the anti-Markovnikov addition products. Sterically hindered and less-hindered, isomerizable and non-isomerizable, aliphatic and aromatic olefins all reacted, suggesting a broad scope of this methodology. Through this investigation, dialkylation

was only observed when coupling the unsubstituted cyclopentanone (**1u**) with ethylene (Fig. 3B); however, we discovered that simply adding water to the reaction enhanced the selectivity for monoalkylation (supplementary materials). For examples exhibited in Fig. 3, B and C, the ketones were employed as the solvents for optimal performance, and the turnover numbers (TON) based on the Rh monomer were used to measure the efficiency of these reactions. Given the volatility of these alkylation products, their accurate yields were determined by gas chromatography (GC) or ^1H NMR analysis; a portion of the pure products could be isolated via silica gel chromatography and fully characterized (supplementary materials), although compounds **3w** and **3x** were identified by comparison of their crude ^1H NMR, GC, and GC-mass spectrometry data with authentic samples.

To gain more mechanistic insight into this bifunctional catalyst-mediated ketone/olefin coupling, we conducted several additional experiments (Fig. 4). First, we isolated a key Rh-enamine complex (**4**) from enamine **5** and $[\text{Rh}(\text{coe})_2\text{Cl}]_2$ (Fig. 4A). The x-ray structure of **4** shows that the azaindoline plane is twisted 62.5° (compared with the x-ray structure of free enamine **5**) to allow chelation of the metal with the pyridine and the olefin, suggesting a preactivated conformation for the subsequent C-H insertion (see step a in Fig. 2). Second, attempts to capture the metal-hydride intermediate incorporating an IMes ligand were unfruitful; however, we successfully isolated and obtained the x-ray structure of Rh-H complex **6** with PMe_3 as the dative ligand (Fig. 4B). Although complex **6** did not react with ethylene, it demonstrated the feasibility of insertion of a low-valent transition metal into enamine vinyl C-H bonds by oxidative addition (see step b in Fig. 2). Third, two deuterium-labeling experiments (Fig. 4C) were carried out to examine the proposed metal-hydride migratory insertion and reductive elimination steps (see steps c and d in Fig. 2). Following the proposed sequence, an α hydrogen of the ketone substrate should be transferred to the terminal position of the ethyl substituent of the alkylation product. Indeed, when the α and α' -deuterated (at the 93% D level) 3-methylcyclopentanone (**1s'**) was subjected to the standard reaction conditions, more than 82% deuterium incorporation was observed at the C2 position of the ethyl group. In addition, a stable conjugated enamine (**8**) could be isolated in good yield through coupling cyclopentanone (**1u'** α and α' -92% deuterated), amine **L1**, and diphenylacetylene. Similarly, significant deuterium incorporation (68%) was observed at the vinyl hydrogen of compound **8**. X-ray diffraction analysis confirmed the E olefin geometry, further supporting a syn-migratory insertion pathway (see step c in Fig. 2). The erosion in deuterium incorporation for both labeling experiments is likely caused by proton exchange with the NH hydrogen of **L1** and/or the protons of $\text{TsOH}\cdot\text{H}_2\text{O}$ (for more details, see the supplementary materials, section 3.6). Altogether, these results are consistent with our proposed mechanism in Fig. 2.

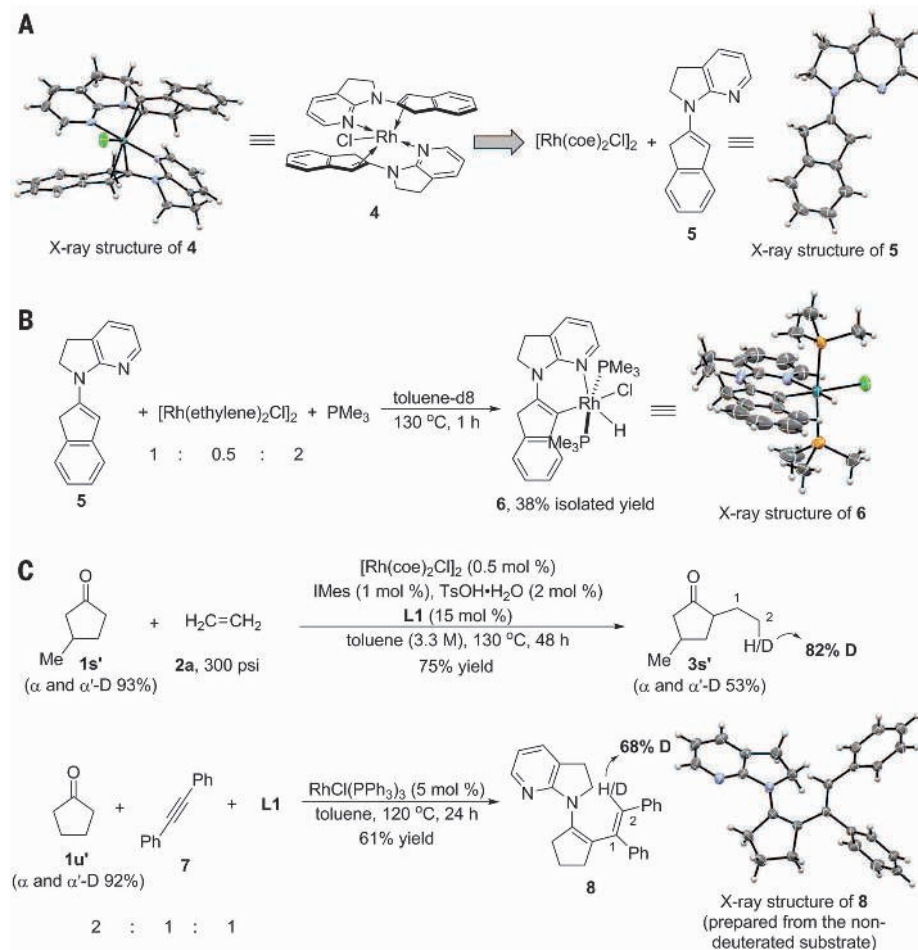


Fig. 4. Preliminary mechanistic studies. (A) Structure of the enamine-Rh complex. (B) Synthesis of a Rh-H complex. (C) Deuterium-labeling experiments.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6192/68/suppl/DC1
Materials and Methods
Supplementary Text
Table S1
References (31–58)

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SEPARATION MEMBRANES

Interfacial microfluidic processing of metal-organic framework hollow fiber membranes

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Molecular sieving metal-organic framework (MOF) membranes have great potential for energy-efficient chemical separations, but a major hurdle is the lack of a scalable and inexpensive membrane fabrication mechanism. We describe a route for processing MOF membranes in polymeric hollow fibers, combining a two-solvent interfacial approach for positional control over membrane formation (at inner and outer surfaces, or in the bulk, of the fibers), a microfluidic approach to replenishment or recycling of reactants, and an in situ module for membrane fabrication and permeation. We fabricated continuous molecular sieving ZIF-8 membranes in single and multiple poly(amide-imide) hollow fibers, with H₂/C₃H₈ and C₃H₆/C₃H₈ separation factors as high as 370 and 12, respectively. We also demonstrate positional control of the ZIF-8 films and characterize the contributions of membrane defects and lumen bypass.

Molecular sieving membranes have created interest as high-performance separation systems for production of petro-based and renewable fuels and chemicals. Compared to thermodynamically driven separation methods such as distillation, membrane-based processes can substantially reduce the energy and capital costs of separating molecules on a large scale. Membranes composed of molecular sieving materials such as zeolites (1), layered zeolites (2), or metal-organic frameworks (MOFs) (3) have intrinsic advantages over polymeric membranes, such as a simultaneously high permeability and selectivity. Despite their performance limitations, polymeric membranes have continued to dominate industrial membrane separations owing to their relative ease of processing into morphologies such as hollow fibers (4). One challenge facing molecular sieving membranes is the lack of an easily scalable, reliable, and benign fabrication process (5–7). Zeolite membranes are further hampered by the need for hydrothermal synthesis on high-cost support materials. MOFs consist of metal centers connected by coordination bonds to organic linker molecules. They have been used to grow crystalline membranes on disk and tubular substrates through techniques similar to those developed for zeolitic membranes (8). The zeolitic imidazolate framework (ZIF) subclass of MOFs is of particular interest for membrane fabrication because of its tunable pore size and chemistry (9) and relatively good thermal and chemical stability (10, 11). In an early demonstration of scalable ZIF membrane processing

(12), we synthesized ZIF-90 membranes by seeded growth on the outer surfaces of porous polymeric poly(amide-imide) (Torlon) hollow fibers of ~250-μm outer diameter by immersion in a methanolic precursor solution at mild conditions (65°C). Free-standing MOF films can also be synthesized at the interfaces between two immiscible solvents (13). However, molecular sieving membranes on the inner surfaces of hollow fibers also have advantages for rapid, scalable fabrication due to the ability to be bundled in close proximity while avoiding membrane-membrane contact points and interfaces that lead to defects during synthesis. Synthesis of selective membranes in microscopic confined spaces faces a number of challenges: reactant availability and transport, positional control of the membrane, and scalability. As the bore size (and hence volume) is decreased to microscopic dimensions, film formation becomes limited by reactant availability and local inhomogeneities (14).

We report a methodology for fabricating molecular sieving MOF membranes (specifically, ZIF-8), which we refer to as interfacial microfluidic membrane processing (IMMP) (Fig. 1). IMMP thus combines three key concepts: (i) in situ ZIF-8 film synthesis in the membrane module (Fig. 1A); (ii) a two-solvent interfacial approach (Fig. 1, B and C) that can be tuned to achieve positional control over membrane formation (at inner and outer surfaces, as well as inside the bulk, of the porous fiber); and (iii) the controlled supply, replenishment, and recycling of reactants at microfluidic conditions in the hollow fiber bore. Our approach can be applied more generally to other MOF materials, but we demonstrate our key findings here with the example of ZIF-8, which has been identified as a promising candidate for important separations such as H₂ from hydrocarbons and propylene from propane (3, 15). To study the IMMP concept, we designed and fabricated a reusable flow module that serves as

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both a membrane fabrication reactor as well as a gas permeation module (fig. S1). Torlon hollow fibers with a pore size of ~ 100 nm and room-temperature N_2 permeance of 53,000 gas permeation units (GPU; $1 \text{ GPU} = 3.348 \times 10^{-10} \text{ mol m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$), produced in-house and characterized as previously described in detail (12, 16), were used as membrane fabrication supports. A dilute zinc nitrate hexahydrate/1-octanol solution was flowed at a typical rate of 10 $\mu\text{l}/\text{hour}$ through the bore of a horizontally mounted Torlon fiber, and a concentrated aqueous 2-methylimidazole (2-mIm) solution was present in the reactor chamber on the shell side (17). Membrane growth was controlled (fig. S2) by the use of continuous bore solution flow conditions, static bore solution conditions, or intermittent conditions (initial continuous bore solution flow followed by static conditions and then flow replenishment of bore

reactant). Static growth conditions produced dense, noncontinuous coatings of ZIF-8 particles in the bore of the fiber (fig. S2). This is due to the lack of sufficient Zn^{2+} ions available in the fiber bore (volume 1.5 μl) to sustain film growth after the initial nucleation and growth of ZIF-8 crystals at the fiber surface. By contrast, a thin membrane ($\sim 2 \mu\text{m}$, fig. S2) was formed via growth under continuous flow without static growth phases. This is due to the relatively rapid transport of reactants to the interface under continuous flow, leading to rapid formation of a ZIF-8 layer. The growing membrane itself becomes a barrier between the two immiscible solvents and confines the liquid-liquid interface to the gaps and interstices between the ZIF-8 crystals. Crystal growth becomes more favorable in these void regions owing to higher reagent accessibility, leading to a more continuous film (13). When the flow profile

was altered to include an initial continuous flow followed by static growth phases interrupted only by short reactant replenishment steps, highly intergrown ZIF-8 membranes of different thicknesses (e.g., $\sim 9 \mu\text{m}$ under the present conditions) were formed (fig. S2). Figure 2A is a lower-magnification image of a ZIF-8 membrane formed under intermittent conditions. Zinc elemental mapping (Fig. 2B) confirms the localization of the membrane to the inner surface of the fiber. To determine the uniformity of membrane formation, we obtained cross sections of the fiber at different locations along the fiber length and measured the membrane thickness at these locations. Figure 2C depicts the membrane thickness measured along the length of the fiber and shows that a continuous membrane of average thickness $8.8 \pm 1.4 \mu\text{m}$ was formed throughout the fiber length (also see fig. S3).

We then hypothesized that controlling the precursor and solvent locations would allow control over the membrane position (inner surface, outer surface, or in the bulk of the fiber). Although we have studied only inner-surface membranes in detail here, demonstration of outer-surface and in-fiber membranes is also of interest. For example, zeolite DDR membranes formed inside (rather than on) porous ceramic supports were previously shown to have better CO_2/CH_4 separation, and this effect was attributed to greater mechanical and thermal stability (18). In separations limited by low permeability, outer-surface membranes may be desired because of the higher surface area. The two ZIF precursors (here, Zn^{2+} ions and 2-mIm) can be supplied in two different solvents (here, water and 1-octanol) on either the inner (bore) or outer (shell) sides of the hollow fiber. This creates eight different reactor operation possibilities. Table S1 lists five of these cases studied so far, with case 1 shown in fig. 2, A and B, and cases 2 to 5 shown in fig. S4. X-ray diffraction confirmed the ZIF-8 crystal structure of the films formed in cases 1 to 5 (fig. S5). We found that the primary factor influencing the position of the film is the location of the Zn^{2+} reactant. The ZIF-8 film forms at (or near) the location of the Zn^{2+} reactant. The Zn^{2+} concentration is 0.018 mol/liter in all cases, whereas the

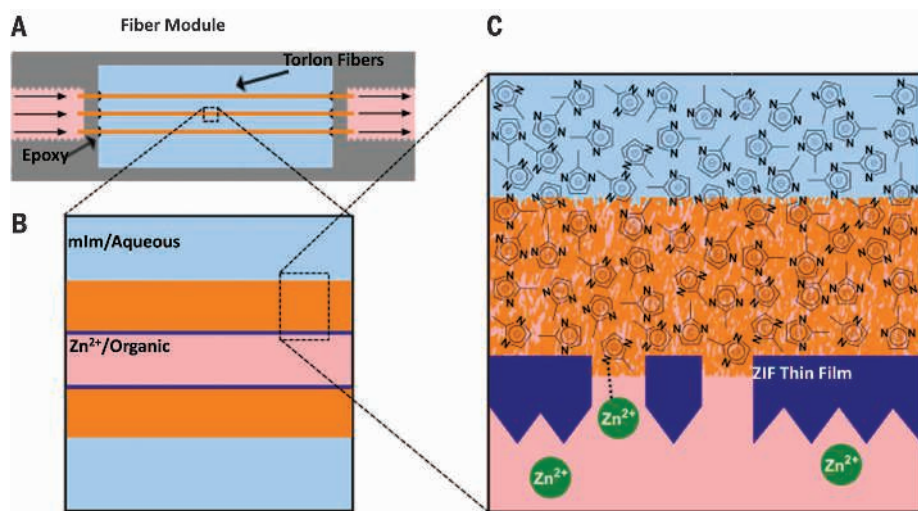
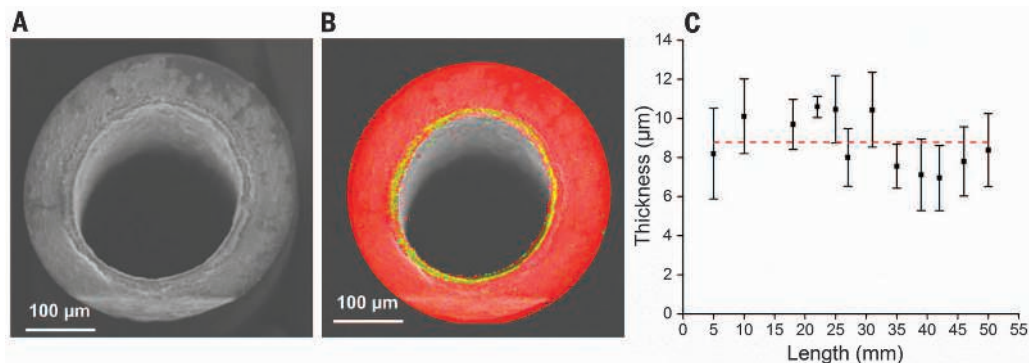


Fig. 1. Scheme depicting the interfacial microfluidic membrane processing (IMMP) approach for MOF membranes in hollow fibers. (A) Side view of a series of fibers mounted in the IMMP reactor. (B) The Zn^{2+} ions are supplied in a 1-octanol solution (light red) flowing through the bore of the fiber, whereas the methylimidazole linkers are supplied on the outer (shell) side of the fiber in an aqueous solution (light blue). (C) Magnified view of fiber support during synthesis. In this example, the membrane forms on the inner surface of the fiber by reaction of the two precursors to form a polycrystalline ZIF-8 layer (dark blue).

Fig. 2. Cross-sectional SEM and EDX characterization of ZIF-8/hollow fiber membrane morphology and thickness. (A) Cross section SEM showing the ZIF-8 membrane on the inner surface of the hollow fiber. (B) EDX elemental maps of carbon (red) and superimposed zinc (green) showing the localization of the ZIF-8 membrane to the inner surface of the fiber. (C) Membrane thickness measured from cross sections obtained at different locations along the 5-cm length of the hollow fiber, showing the formation of a continuous membrane of essentially uniform thickness. The average membrane thickness along the entire fiber (red dashed line) is $8.8 \pm 1.4 \mu\text{m}$. The thickness at each location was measured from five randomly chosen points along the circumference of the hollow fiber cross section. Figure S3 shows several examples of the membrane cross sections taken at different distances along the fiber.



2-mIm concentration is 1.37 mol/liter and hence in large excess (table S1). Assuming that the diffusion rate of reactants to the film surface limits the growth of the membrane (i.e., the reactants are quickly consumed by chemical reaction upon encountering each other), and considering that the diffusivities of the two precursors in the liquid solvents are of the same order of magnitude, the ratio of distances of the membrane from the two bulk solutions will be proportional to the ratio of the bulk concentrations of the two reactants (table S1). This is in agreement with the results of detailed multiscale modeling of film formation in opposing-reactant geometries (19), wherein the ratio of reactant concentrations is identified as the most important parameter. In all five cases, the bore side reactant is first introduced, filling the fiber bore and also infiltrating the porous support, before the shell side reactant is introduced. In cases 1, 4, and 5, the two solvents are immiscible and form a sharp interface (e.g., Fig. 1C), but the precursors are soluble in both solvents and can diffuse between them. In cases 4 and 5, the limiting Zn^{2+} reactant is introduced on the shell side and hence the membrane forms on the outer surface. In case 1, although Zn^{2+} ions are initially present throughout the porous fiber and can react with the 2-mIm linker molecules diffusing from the shell side, the successful nucleation and growth of ZIF-8 only occurs when there is a sufficiently replenished supply of Zn^{2+} , i.e., on the bore side. This is again in agreement with the modeling predictions of the role of initial conditions in determining the location of solid nucleation (19). In cases 2 and 3, where the two solvents are identical and Zn^{2+} is introduced on the bore side, the film again forms at the bore side, but about 10 μm into the porous fiber. The miscibility of the two solvents likely alters the transport rate of the limiting Zn^{2+} reactant toward the shell side, thus moving the location of ZIF-8 nucleation a short distance into the porous support.

We characterized the hydrogen (H_2) and propylene (C_3H_6) separation properties of the case-1 ZIF-8 membrane formed under intermittent flow conditions by $\text{H}_2/\text{C}_3\text{H}_8$ and $\text{C}_3\text{H}_6/\text{C}_3\text{H}_8$ equimolar mixture permeation as a function of temperature, with the IMMP reactor directly acting as a permeation module (fig. S6). The as-made ZIF-8 membranes exhibited clear molecular sieving properties: high H_2 permeances, sharp $\text{H}_2/\text{C}_3\text{H}_8$ separation factors as high as 130 ± 8 at 120°C , and strong temperature dependence of H_2 permeance, indicating activated molecular transport through the ZIF-8 pores (Fig. 3, A and B). However, the C_3H_8 permeance was much larger than that expected from previous studies (8, 20–23) and prevented a high $\text{C}_3\text{H}_6/\text{C}_3\text{H}_8$ separation factor (2.2 ± 0.4 at 25°C). This was hypothesized to result from both molecular transport through the ZIF-8 membrane and from bypassing (24) of the ZIF-8 membrane by the feed molecules through the ends of the fiber (fig. S7). To suppress this membrane bypass, we included a lumen-capping step to the IMMP, accomplished by applying a controlled amount of a solution con-

taining poly(dimethylsiloxane) (PDMS) to the ends of the mounted module. The PDMS solution was readily absorbed by capillary action at the fiber ends, blocking the 100-nm pores of the fiber and leading to bypass suppression. Scanning electron micrograph (SEM) imaging and energy-dispersive x-ray (EDX) analysis showed that the porous fiber was infiltrated by PDMS, whereas the bore remained unblocked (fig. S8). PDMS infiltration into the fiber was also profiled with EDX mapping of the Si/Zn ratio along the hollow fiber length (fig. S9), showing that the infiltration depth was 8 mm from the fiber ends. Because the permeances of the gases through PDMS and ZIF-8 (25–27) are three orders of magnitude lower than through the bare porous fiber, the C_3H_8 flux should decrease substantially after capping. The PDMS-sealed fibers showed much higher $\text{H}_2/\text{C}_3\text{H}_8$ and $\text{C}_3\text{H}_6/\text{C}_3\text{H}_8$ separation factors (370 ± 60 at 120°C and 12 ± 3 at 25°C , respectively) (Fig. 3, A and B, and table S2). This is consistent with previously reported ZIF-8 membranes with low defect densities (8, 20–23). Notably, the C_3H_8 permeance decreased by a factor of 10 after capping, showing that most of the earlier observed C_3H_8 flux was due to bypassing and that the capping step largely shuts down this nonselective path. The permeate streams

after capping contain essentially pure H_2 , or 92% $\text{C}_3\text{H}_6/8\%$ C_3H_8 , which are substantial upgrades from the equimolar feeds. Longer-term $\text{H}_2/\text{C}_3\text{H}_8$ and $\text{C}_3\text{H}_6/\text{C}_3\text{H}_8$ permeation measurements carried out over a period of 35 days showed the high stability of the ZIF-8 membrane (figs. S10 and S11).

To reliably quantify the efficacy of the lumen-capping step and the contribution of membrane defects such as grain boundaries or microcracks, we conducted single-component measurements on four gases (C_3H_6 , C_3H_8 , $n\text{-C}_4\text{H}_{10}$, $i\text{-C}_4\text{H}_{10}$) at 25°C using as-made and sealed membranes (table S3). The results were fitted to a permeation model (17) that yields the relative molar flow contributions of the ZIF-8 membrane, membrane defects, and bypass for each gas. The PDMS capping step is seen to be highly effective in closing the bypass path (table S4). In the as-made membranes, about 60% of the molar flow of C_3H_8 occurs through bypass, about 37% through membrane defects, and 3% through ZIF-8. After PDMS capping, the contribution of bypass is essentially eliminated, and about 92% of the small remaining C_3H_8 flow (~ 2 GPU of permeance) is through membrane defects. This indicates that for the $\text{C}_3\text{H}_6/\text{C}_3\text{H}_8$ separation case, optimization of ZIF-8 IMMP could focus on further reduction of defect permeance by variation of growth conditions

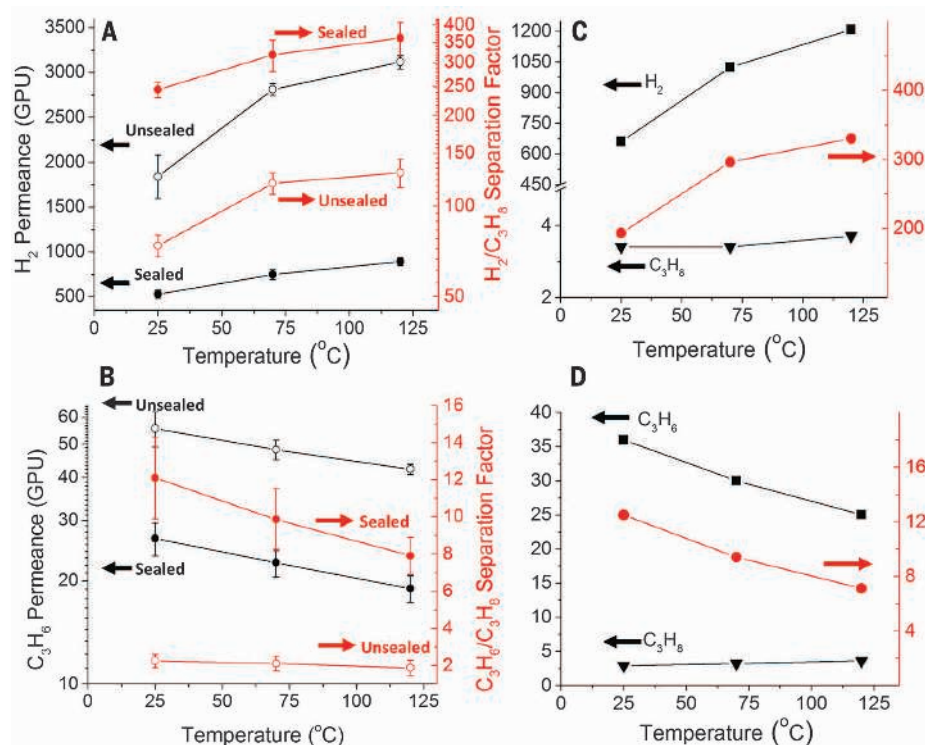


Fig. 3. Gas permeation properties of ZIF-8/hollow fiber membranes fabricated by IMMP in binary $\text{H}_2/\text{C}_3\text{H}_8$ and $\text{C}_3\text{H}_6/\text{C}_3\text{H}_8$ separations with equimolar feed mixtures. (A) Binary $\text{H}_2/\text{C}_3\text{H}_8$ and (B) binary $\text{C}_3\text{H}_6/\text{C}_3\text{H}_8$ separation characteristics (permeances and separation factors) of single-fiber membranes as a function of temperature, before and after lumen sealing with PDMS. Lumen sealing leads to a large increase in separation factor in both cases. (C) and (D) depict the same performance characteristics obtained from a module of three fibers processed simultaneously with IMMP, showing separation factors similar to those of the single-fiber membranes. The error bars in (A) and (B) were estimated from three independently synthesized membrane samples, and the numerical data for all of the lumen-sealed membranes are shown in table S2.

or by postsynthesis treatments (28, 29). By contrast, separations such as H_2/C_3H_8 that involve a fast-permeating species are not appreciably affected by membrane defects. IMMP is also inherently a modular and parallel approach that should allow independent and simultaneous processing of membranes in multiple fibers. To test this hypothesis, we applied IMMP to the simultaneous processing of three hollow fibers. The total bore flow rate was increased by a factor of 3 so that the flow rate through individual fibers was maintained. The ends of the module were capped with PDMS, as described earlier. Figure 3, C and D, shows that the H_2/C_3H_8 and C_3H_6/C_3H_8 separation behavior is essentially identical to the single-fiber case, demonstrating the potential for scalability of IMMP. Given the overall importance of tunable ZIF materials for a range of hydrocarbon and light-gas separations, the membrane-processing approach reported here overcomes many limitations of current processes and is a notable step toward realizing scalable molecular sieving MOF membranes.

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F.R. Permeation modeling was carried out by S.N. and A.J.B. All authors contributed to manuscript writing and editing. We thank W. Qiu, R. P. Lively, and A. Rowan (all at Georgia Institute of Technology) for helpful discussions. The Supplementary Materials includes a detailed description of materials and methods, details of the IMMP reactor, time-dependent flow profiles and synthesis cases, SEM images of ZIF-8 membranes, XRD patterns of membranes, schematics of permeation apparatus and gas bypass effects, EDX mapping of the ZIF-8 membrane, permeation modeling equations, and gas permeation data. A patent application related to this work has been filed [U.S. patent application 61/820,489, filed 7 May 2013; S. Nair *et al.*, Flow processing

and characterization of metal-organic framework (MOF) membranes in tubular and hollow fiber modules].

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6192/72/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S11
Tables S1 to S4

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SOCIAL PSYCHOLOGY

Just think: The challenges of the disengaged mind

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In 11 studies, we found that participants typically did not enjoy spending 6 to 15 minutes in a room by themselves with nothing to do but think, that they enjoyed doing mundane external activities much more, and that many preferred to administer electric shocks to themselves instead of being left alone with their thoughts. Most people seem to prefer to be doing something rather than nothing, even if that something is negative.

“The mind is its own place, and in it self/
Can make a Heav’n of Hell, a Hell of Heav’n.”

– John Milton, *Paradise Lost*

The ability to engage in directed conscious thought is an integral part—perhaps even a defining part—of what makes us human. Unique among the species, we have the ability to sit and mentally detach ourselves from our surroundings and travel inward, recalling the past, envisioning the future, and imagining worlds that have never existed. Neural activity during such inward-directed thought, called default-mode processing, has been the focus of a great deal of attention in recent years, and researchers have speculated about its possible functions (1–5). Two related questions, however, have been overlooked: Do people choose to put themselves in default mode by disengaging from the external world? And when they are in this mode, is it a pleasing experience?

Recent survey results suggest that the answer to the first question is “not very often.” Ninety-five percent of American adults reported that they did at least one leisure activity in the past 24 hours, such as watching television, socializing, or reading for pleasure, but 83% reported they spent no time whatsoever “relaxing or thinking” (6). Is this because people do not enjoy having nothing to do but think?

Almost all previous research on daydreaming and mind wandering has focused on task-

unrelated thought, namely cases in which people are trying to attend to an external task (such as reading a book), but their minds wander involuntarily (7, 8). In such cases, people tend to be happier when their minds are engaged in what they are doing, instead of having wandered away (9, 10). A case could be made that it is easier for people to steer their thoughts in pleasant directions when the external world is not competing for their attention. We suggest, to the contrary, that it is surprisingly difficult to think in enjoyable ways even in the absence of competing external demands.

To address these questions, we conducted studies in which college-student participants spent time by themselves in an unadorned room (for 6 to 15 min, depending on the study) after storing all of their belongings, including cell phones and writing implements. They were typically asked to spend the time entertaining themselves with their thoughts, with the only rules being that they should remain in their seats and stay awake. After this “thinking period,” participants answered questions about how enjoyable the experience was, how hard it was to concentrate, etc.

Table 1 summarizes the results of six studies that followed this procedure. Most participants reported that it was difficult to concentrate (57.5% responded at or above the midpoint of the point scale) and that their mind wandered (89.0% responded at or above the midpoint of the scale), even though there was nothing competing for their attention. And on average, participants did not enjoy the experience very much: 49.3% reported enjoyment that was at or below the midpoint of the scale.

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Perhaps the unfamiliar environs of the psychological laboratory made it difficult for people to become lost in and enjoy their thoughts. In study 7, we instructed college-student participants to complete the study at home, by clicking on a link to a Web program when they were alone and free of external distractions. Many participants found it difficult to follow these instructions: 32% reported that they had “cheated” by engaging in an external activity (such as listening to music or consulting their cell phones) or getting up out of their chair. Furthermore, there was no evidence that participants enjoyed the experience more when they were in the privacy of their homes. The mean reported enjoyment was lower when they were at home than when they were in the laboratory [$t(188) = 2.47, P = 0.014$], and participants reported that it was harder to concentrate on their thoughts when they were at home [$t(188) = 2.87, P = 0.005$] (Table 1). These differences must be interpreted with caution, because we did not randomly assign participants to a location, but they suggest that just thinking is no easier at home than it is in the laboratory.

Would participants enjoy themselves more if they had something to do? In study 8, we randomly assigned participants to entertain themselves with their own thoughts or to engage in external activities (such as reading a book, listening to music, or surfing the Web). We asked the latter participants not to communicate with others (e.g., via texting or emailing), so that we could compare nonsocial external activities (such as reading) with a nonsocial internal activity (thinking). As seen in Table 1, participants enjoyed the external activities much more than just thinking [$t(28) = 4.83, P < 0.001$], found it easier to concentrate [$t(28) = 4.16, P < 0.001$], and reported that their minds wandered less [$t(28) = 3.61, P = 0.001$].

To see whether the difficulty with “just thinking” is distinctive to college students, in study 9 we recruited community participants at a farmer’s market and a local church. The participants ranged in age from 18 to 77 (median age = 48.0 years). As in study 7, they completed the study online in their own homes, after receiving instructions to do so when they were alone and free of any external distractions. The results were similar to those found with college students. There was no evidence that enjoyment of the thinking period was related to participants’ age, education, income, or the frequency with which they used smart phones or social media (table S2).

There was variation in enjoyment in our studies, and we included several individual difference measures to investigate what sort of person enjoys thinking the most (summarized in table S3). The variables that consistently predicted enjoyment across studies were items from two subscales of the Short Imaginal Process Inventory (11). The Positive Constructive Daydreaming subscale (e.g., “My daydreams often leave me with a warm, happy feeling”) correlated positively with enjoyment, and the Poor Attentional Control subscale (e.g., “I tend to be

easily bored”) correlated negatively with enjoyment. None of the other correlations exceeded 0.27 (table S3).

So far, we have seen that most people do not enjoy “just thinking” and clearly prefer having something else to do. But would they rather do an unpleasant activity than no activity at all? In study 10, participants received the same instructions to entertain themselves with their thoughts in the laboratory but also had the opportunity to experience negative stimulation (an electric shock) if they so desired. In part 1 of the study, participants rated the pleasantness of several positive stimuli (e.g., attractive photographs) and negative stimuli (e.g., an electric shock). Participants also reported how much they would pay to experience or not experience each stimulus again, if they were given \$5. Next, participants received our standard instructions to entertain themselves with their thoughts (in this case for 15 min). If they wanted, they learned, they could receive an electric shock again during the thinking period by pressing a button. We went to some length to explain that the primary goal was to entertain themselves with their thoughts and that the decision to receive a shock was entirely up to them.

Many participants elected to receive negative stimulation over no stimulation—especially men: 67% of men (12 of 18) gave themselves at least one shock during the thinking period [range = 0 to 4 shocks, mean (M) = 1.47, SD = 1.46, not including one outlier who administered 190 shocks to himself], compared to 25% of women (6 of 24; range = 0 to 9 shocks, $M = 1.00$, SD = 2.32). Note that these results only include participants who had reported that they would pay to avoid being shocked again. (See the supplementary materials for more details.) The gender difference is probably due to the tendency for men to be higher in sensation-seeking (12). But what is striking is that simply being alone with their own thoughts for 15 min was apparently so aversive that it drove many participants to self-administer an electric shock that they had earlier said they would pay to avoid.

Why was thinking so difficult and unpleasant? One possibility is that when left alone with their thoughts, participants focused on their own shortcomings and got caught in ruminative thought cycles (13–16). Research shows, however, that self-focus does not invariably lead to rumination (17), a finding that was confirmed in our studies. At the conclusion of the thinking period, we asked participants to describe what they had been thinking about, and we analyzed these reports with linguistic analysis software (18). There was no relationship between the extent of self-focus (as assessed by the use of first-person personal pronouns) and participants’ use of positive-emotion words, negative-emotion words, or reported enjoyment of the thinking period correlations = 0.033, 0.025, and 0.022, respectively; 218 participants, ns) (see table S4 for other results of the linguistic analyses).

Another reason why participants might have found thinking to be difficult is that they simultaneously had to be a “script writer” and an “experiencer”; that is, they had to choose a topic to think about (“I’ll focus on my upcoming summer vacation”), decide what would happen (“Okay, I’ve arrived at the beach, I guess I’ll lie in the sun for a bit before going for a swim”), and then mentally experience those actions. Perhaps people would find it easier to enjoy their thoughts if they had time to plan in advance what they would think about. We tested this hypothesis in studies 1 to 7. Participants were randomly assigned to our standard “thinking period” condition (the results of which are shown in Table 1) or to conditions in which they first spent a few minutes planning what they would think about. We tried several versions of these “prompted fantasy” instructions (summarized in table S1) and found that none reliably increased participants’ enjoyment of the thinking period. Averaged across studies, participants in the prompted fantasy conditions reported similar levels of enjoyment as did participants in the standard conditions [$M = 4.97$ versus 4.94 (SDs = 1.80, 1.84), $t(450) = 0.15$, ns].

There is no doubt that people are sometimes absorbed by interesting ideas, exciting fantasies,

Table 1. Reactions to the “thinking period” under different conditions.

Measure		Studies 1 to 6: In the lab (<i>n</i> = 146)	Study 7: At home (<i>n</i> = 44)	Study 8: At home	
				Standard thought instructions (<i>n</i> = 15)	External activities (<i>n</i> = 15)
Enjoyment*	SD	1.77	1.95	2.23	1.91
	<i>M</i>	5.12	4.35	3.20	6.87
Hard to concentrate†	SD	2.23	1.72	2.28	2.01
	<i>M</i>	5.04	6.09	6.07	2.80
Mind wandering‡	SD	1.92	1.85	1.80	2.66
	<i>M</i>	6.86	7.14	6.67	3.67

*Mean of three items, each answered on nine-point scales: How enjoyable and entertaining the thinking period was and how bored participants were (reverse-scored). Cronbach’s alpha = 0.89. †Extent to which participants reported that it was hard to concentrate on what they chose to think about (nine-point scale; the higher the number, the greater the reported difficulty). ‡Extent to which participants reported that their mind wandered during the thinking period (nine-point scale; the higher the number, the greater the reported mind-wandering).

and pleasant daydreams (19–21). Research has shown that minds are difficult to control (8, 22), however, and it may be particularly hard to steer our thoughts in pleasant directions and keep them there. This may be why many people seek to gain better control of their thoughts with meditation and other techniques, with clear benefits (23–27). Without such training, people prefer doing to thinking, even if what they are doing is so unpleasant that they would normally pay to avoid it. The untutored mind does not like to be alone with itself.

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SUPPLEMENTARY MATERIALS

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Materials and Methods

Additional Analyses across Studies

Fig. S1

Tables S1 to S4

References (28–40)

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CLIMATE CHANGE

Climate change and wind intensification in coastal upwelling ecosystems

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In 1990, Andrew Bakun proposed that increasing greenhouse gas concentrations would force intensification of upwelling-favorable winds in eastern boundary current systems that contribute substantial services to society. Because there is considerable disagreement about whether contemporary wind trends support Bakun's hypothesis, we performed a meta-analysis of the literature on upwelling-favorable wind intensification. The preponderance of published analyses suggests that winds have intensified in the California, Benguela, and Humboldt upwelling systems and weakened in the Iberian system over time scales ranging up to 60 years; wind change is equivocal in the Canary system. Stronger intensification signals are observed at higher latitudes, consistent with the warming pattern associated with climate change. Overall, reported changes in coastal winds, although subtle and spatially variable, support Bakun's hypothesis of upwelling intensification in eastern boundary current systems.

In eastern boundary current systems (EBCSs), coastal upwelling fuels high productivity, supporting vast and diverse marine populations. With a surface area of only ~2% of the global oceans, EBCSs provide upward of 20% of wild marine-capture fisheries (1) as well as essential habitat for marine biodiversity (2). Understanding upwelling variability is also key to assessments of marine ecosystem health, influencing factors such as ocean acidification and deoxygenation (3–5). Although the ecological relevance of upwelling is clear, the future of upwelling under anthropogenic climate change is not (6–8). In 1990, Andrew Bakun hypothesized that global warming could result in steeper temperature and sea-level pressure gradients between the oceans and the continents, causing alongshore upwelling-favorable winds to intensify (6). Although the increase in global temperatures is unquestioned (7), its influence on upwelling-favorable winds remains uncertain. In an attempt to resolve disagreement in the literature concerning the intensification of upwelling winds, we conducted a “preponderance of evidence” meta-analysis on results from previous studies that tested Bakun's wind intensi-

fication hypothesis. Our meta-analysis focused on the outcome of Bakun's purported mechanism: upwelling-favorable wind intensification over the past 6+ decades.

We synthesized results from 22 studies published between 1990 and 2012, 18 of which contained quantitative information on wind trends. Our resulting database contains 187 non-independent wind trend analyses based on time series ranging in duration from 17 to 61 years [tables S1 to S3 (9)]. We tested whether the evidence from these studies was consistent (increasing winds) or inconsistent (weakening winds) with the Bakun hypothesis. Bakun proposed that winds would intensify in the upwelling or warm season; i.e., May to August in the Northern Hemisphere and November to February in the Southern Hemisphere. Therefore, we categorized each trend based on the months averaged for its calculation: “warm season” or “annual” (all months). Bakun surmised that there would be latitudinal variation in wind trends and predicted that the most substantial intensification would be in the “core” of each EBCS. Therefore, to test for spatial heterogeneity in wind trends, we included absolute latitude in our models (9). We compared results from observational data and model-data re-analysis products, because previous research has shown different trends among these data types (10, 11).

We used logistic regression to model the consistency of wind trends with the Bakun hypothesis. Although all studies included in our analysis undertook formal statistical analysis, they used different analyses and statistical approaches and also used a range of significance levels (0.01 to 0.10), many of which were reported only categorically (9). Consequently, we used a qualitative approach (table S3) in which we down-weighted nominally nonsignificant trends to half the weight

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of nominally significant trends. We restricted logistic regression models to two simultaneous explanatory variables per model, one of which was “ecosystem.” We did not attempt to adjust for non-independence of related coastal winds time series; caution is therefore warranted in interpreting the results of our meta-analysis.

Classically, four EBCs are recognized (Fig. 1), but because upwelling-favorable winds are driven by different continental pressure systems in the Iberian and North African regions of the Canary Current (11, 12), we separated these regions for analysis. Overall, we found that published papers support wind intensification in three out of five upwelling ecosystems over the past six decades. For the California and Humboldt systems, warm-season analyses were significantly more likely to show wind intensification (i.e., log-odds ratios > 0 ; $P < 0.001$; table S5) than were annually summarized data. For the Canary ($P = 0.170$) and Iberian ($P = 0.357$) systems, warm-season winds were as likely to be intensifying as not (table S5). Studies in the Benguela system, although exclusively based on annually summarized data, reflected a greater likelihood of intensifying winds ($P = 0.011$). Studies of annually summarized data in other systems failed to detect wind intensification (Fig. 1). In contrast, annual data in the Canary ($P = 0.018$) and Iberian ($P < 0.001$) systems showed greater

likelihood of weakening than of strengthening wind patterns. Overall, the likelihood of detecting a positive trend in winds was 5.9 times higher for warm-season data than for annual data ($P < 0.001$; table S5).

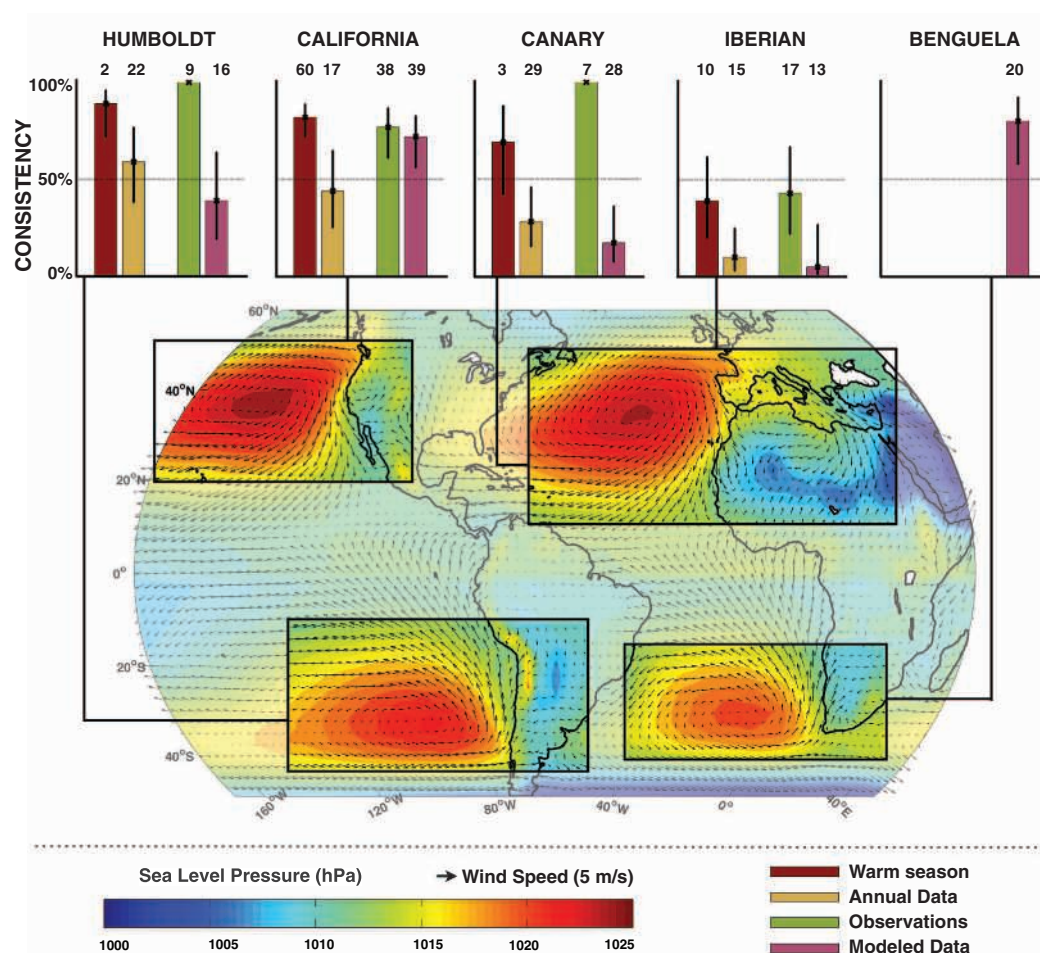
The probability of detecting intensifying winds was dependent on data type (tables S4 and S5; Benguela excluded because all data are model-data reanalysis products). Studies based on direct observations were significantly more likely to detect intensifying than weakening winds for the California ($P = 0.002$), Humboldt ($P < 0.001$), and Canary ($P < 0.001$) systems, as were studies of model-data reanalysis winds in the California system ($P = 0.009$). In the Iberian and Canary systems, model-data reanalysis products were more likely to detect weakening winds ($P = 0.004$ and $P = 0.002$, respectively). Because there were three times more annual than seasonal studies using model-data reanalysis products and almost four times more seasonal than annual studies using observational data, these findings concerning season and data type are not independent (9).

Within systems, the likelihood of a study detecting wind intensification increased significantly ($P = 0.004$) with absolute latitude (Fig. 2 and tables S4 and S5). Wind intensification was found across most latitudes for the Benguela (pole-

ward of 20°S) and California systems (poleward of 32.5°N), as well as over the southern portion (poleward of 14°S) of the Humboldt (Fig. 2) system. Weakening winds were found at most latitudes for the Iberian system and over the southern half (equatorward of 23°N) of the Canary system.

To test the sensitivity of the conclusions to our treatment of nonsignificant trends, we reexamined the data after categorizing all nonsignificant trends as opposed to the wind intensification hypothesis. Results of this conservative approach remove support for long-term increases in upwelling intensity (fig. S1). However, we consider our original approach (categorizing all positive trends as supportive of the wind intensification hypothesis and a priori down-weighting nonsignificant trends) to be most appropriate, because nonsignificant trends contain information, albeit with less confidence than significant trends. Further sensitivity tests show that (i) categorizing nonsignificant trends as equivocal (i.e., essentially removing these trends from the meta-analysis) or (ii) equal weighting across significant and nonsignificant trends (tables S6 and S7) produces results that support our a priori approach of down-weighting nonsignificant trends [fig. S1 (9)]. In summary, based on our assumptions and approach, observational and warm-season

Fig. 1. EBCs of the world showing warm-season spatial climatologies of sea-level pressure and surface wind fields based on the NCEP/NCAR (National Centers for Environmental Prediction/National Center for Atmospheric Research) model-data reanalysis product. These are estimates from logistic regression of consistency with the wind intensification hypothesis; bars show the estimated probability ($\pm 95\%$ confidence intervals). Numbers above the bars denote the number of trends contributing to each point estimate and confidence interval. The dashed horizontal line denotes the null hypothesis of equal probability of increasing or decreasing winds.



data provide support for wind intensification in the California and Humboldt systems, as do model-data reanalysis products from the Benguela system and observational time series from the Canary system (11). Results for the Iberian system were contrary to the hypothesis, with contemporary winds weakening, corroborated by one of the recent studies published after our meta-analysis was completed (12). In general, annually averaged wind trends do not support intensification, highlighting that some of the disagreement in previous studies can be resolved by considering winds during warm (upwelling) seasons only, as originally specified by Bakun.

Model-data reanalyses and observational data showed some contradictory results. An exception was found for the California system, where both data types supported the hypothesis. This is probably due to the high density of observational records included in this well-measured system (9, 10, 13). Changes in instrumentation and time period covered, as well as natural (interannual and lower-frequency) climate variability,

may be sources of bias in our meta-analysis. Most time series examined (91%) cover the period from the late 1940s to mid-2000s. By integrating almost 200 trend analyses, the sensitivity of our results to natural interannual variability and changes in instrumentation may have been reduced. The choices of study period and duration (9) nonetheless remain sources of uncertainty. We examined study duration in some detail (tables S4 and S5), but results were equivocal. In the Pacific systems, study duration had little effect on reported wind trends. In the Atlantic sector, there was a negative relationship between reported trends and study duration (fig. S2). It is clear that study duration and period of study are both unresolved factors that contribute to uncertainty in documented patterns of change both in the literature and in this meta-analysis. Variations in study duration and period also complicate the attribution of wind trends to anthropogenic climate change versus low-frequency climate variability, which influences wind intensity at interannual to multidecadal scales (14, 15). The time series

amassed to date and analyzed here are short relative to the time frame needed to differentiate between natural climate variability and anthropogenic forcing. Furthermore, the paucity of spatially comprehensive observations challenges attempts to clearly distinguish the impact of natural low-frequency variability from anthropogenic change. These are issues that require further investigation as longer coastal-wind time series become available.

In addition to variation among systems, we found an increased likelihood of wind intensification at higher latitudes within most systems. This may reflect stronger warming trends observed toward the poles than the equator (16). An exception is the Iberian system, which is driven by a relatively weak continental low over Europe. It is possible that further analyses of wind trends at subannual scales in that system might reveal different trends than those described here, because the region is strongly influenced by the North Atlantic Oscillation (NAO), although the NAO may affect coastal upwelling there most in the fall and winter (11, 12, 15, 17).

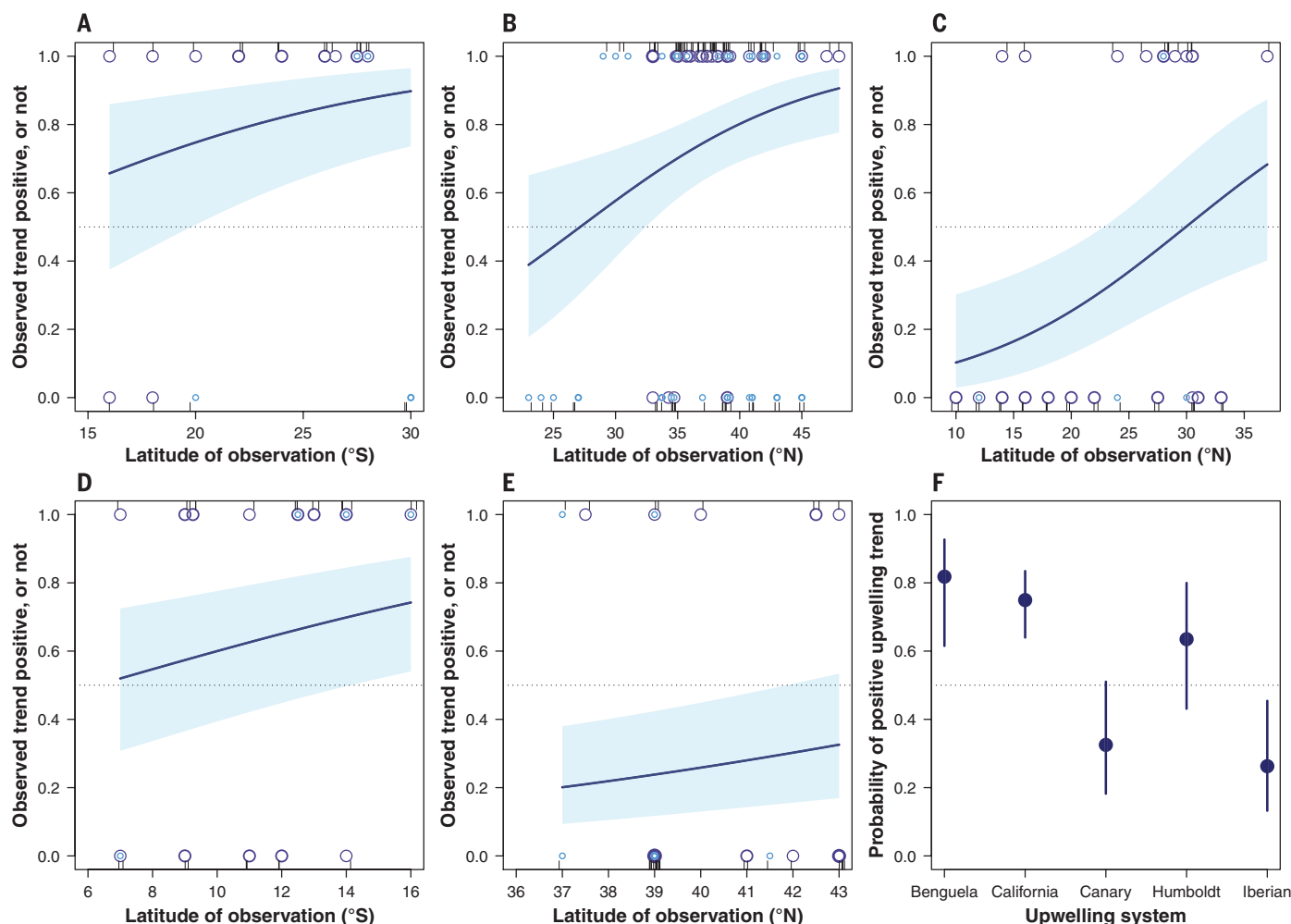


Fig. 2. Effect of absolute latitude by system. (A) Benguela, (B) California, (C) Canary, (D) Humboldt, (E) Iberian. Open circles represent trends in wind time series as reported in the literature (0 = weakening; 1 = strengthening); nonsignificant trends (light blue) are scaled to half the size of significant trends (dark blue). The weight of the circle outline is scaled to sample size at a given latitude. Tick marks

adjacent to each circle indicate the latitude of each data set, with slight adjustments to emphasize the number of observations at any given point. The solid line represents the model fit; the shaded area is the asymptotic 95% confidence envelope. (F) Consistency with wind intensification (including 95% confidence intervals), as calculated across all latitudes within each of the five ecosystems.

Wind intensification in the California, Benguela, and Humboldt ecosystems could benefit marine populations by increasing nutrient input into subtropical euphotic zones if primary production is nutrient-limited. Large increases in wind strength, however, could be detrimental by disrupting trophic interactions (18), causing transport of planktonic organisms off continental shelves (19), or increasing acidic or hypoxic waters in shelf habitats (4, 5). Although positive trends in coastal chlorophyll-a concentration off California and elsewhere (20) conform to the wind trends described here, there is no reason to assume that these changes will translate into increases in productivity across intermediate and higher trophic levels, although a recent study linked wind increases to improvements in albatross foraging efficiency and productivity (21). If new primary production constrains fisheries (22), greater primary productivity could enhance food production. However, ocean warming could counter stronger upwelling by increasing stratification (23), making it difficult to forecast ecological responses. Moreover, given the sensitivities of different species to the seasonality of upwelling (24, 25), population variability might be linked to shifts in the phenology of upwelling as much as to changes in amplitude (26). Changes in the phenology of upwelling-favorable winds may also affect the trends within and across ecosystems described here. Ultimately, the sensitivity of observed wind trends to latitude, data type, season, and time-series duration demonstrated in this meta-analysis highlights the need for sustained high-quality observations of coastal winds and emphasizes the complexity of forecasting the consequences of wind intensification for ecosystems.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6192/77/suppl/DC1
Materials and Methods
Figs. S1 and S2
Tables S1 to S7
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EARTHQUAKE DYNAMICS

Mapping pressurized volcanic fluids from induced crustal seismic velocity drops

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Volcanic eruptions are caused by the release of pressure that has accumulated due to hot volcanic fluids at depth. Here, we show that the extent of the regions affected by pressurized fluids can be imaged through the measurement of their response to transient stress perturbations. We used records of seismic noise from the Japanese Hi-net seismic network to measure the crustal seismic velocity changes below volcanic regions caused by the 2011 moment magnitude (M_w) 9.0 Tohoku-Oki earthquake. We interpret coseismic crustal seismic velocity reductions as related to the mechanical weakening of the pressurized crust by the dynamic stress associated with the seismic waves. We suggest, therefore, that mapping seismic velocity susceptibility to dynamic stress perturbations can be used for the imaging and characterization of volcanic systems.

Large volcanic eruptions are preceded by long-term pressure buildup in volcano magmatic and hydrothermal systems. Therefore, knowledge of the extent and state of these pressurized volcanic fluids at depth will help in the better anticipation of future eruptions. In particular, seismic tomography is often used to delineate volcano-feeding systems at different scales (1, 2). However, a major difficulty of traditional seismic imaging of volcanoes is that the geological contrasts of the host rock might dominate the final tomographic images, which are only partially sensitive to the content and state of volcanic fluids (3).

Recent geodetic observations have shown that volcanic areas are characterized by anomalous responses to crustal deformation induced by large earthquakes, as demonstrated by the subsidence of volcanoes in Chile and Japan after the 2010 Maule and 2011 Tohoku-Oki earthquakes (4, 5). This sensitivity to strong coseismic deformation

and shaking is probably associated with the presence of pressurized hydrothermal and magmatic fluids at depth in a fractured medium. We explored the responses of volcanoes to transient stress perturbations by investigating the temporal evolution of crustal seismic velocities in Japan in response to the seismic shaking and deformation caused by the March 2011 moment magnitude (M_w) 9.0 Tohoku-Oki earthquake.

The Hi-net, Japanese high-sensitivity seismograph network, is among the densest in the world; thus, the 2011 Tohoku-Oki earthquake remains the best-recorded large earthquake to date. It was associated with large, widespread static ground deformation and ground shaking (Fig. 1). In this study, we used seismic noise-based monitoring (6) to characterize the response of the upper crust to the coseismic shaking and deformation caused by the earthquake. We analyzed 1 year of continuous seismic records from a portion of the dense Hi-net seismic network (600 stations, as shown in the inset to Fig. 1A), spanning from 6 months before to 6 months after the earthquake occurrence.

We computed the daily vertical-vertical noise cross-correlation functions using a processing scheme that minimized the effects of the strong aftershock activity that followed the Tohoku-Oki

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earthquake (7). To avoid the choice of an arbitrary reference cross-correlation function and to improve the precision of the measurements,

we separately computed velocity changes for all of the possible daily cross-correlation functions for each station pair. Using a Bayesian

least-squares inversion, we retrieved accurate daily continuous velocity change time series for every station pair (7).

We computed the seismic velocity changes averaged over the day of the Tohoku-Oki earthquake and 4 days after, relative to the seismic velocity changes time series averaged over 6 months before the Tohoku-Oki earthquake (Fig. 2A) (7). These changes mainly correspond to the response of the upper crust to the coseismic shaking and deformation. Similar to previous studies of coseismic velocity variations (6, 8), a reduction in velocity was widespread over Honshu Island. Furthermore, the strongest velocity drops were not observed in the area closest to the epicenter or within large sedimentary basins, as would be expected. The patterns of the observed velocity reductions did not correlate with the intensity of the ground shaking or with the coseismic deformation (Fig. 1); instead, the strongest coseismic velocity reductions occurred under volcanic regions. In particular, a large part of the Tohoku volcanic front and the Mt. Fuji volcanic region are well delineated.

The mechanism by which seismic velocities decrease in response to stress perturbations is commonly described as related to the opening of cracks (9, 10), which might also induce an increase in permeability and a transfer of fluids at depth and may lead to further triggering of earthquakes. Over long distances, large earthquakes

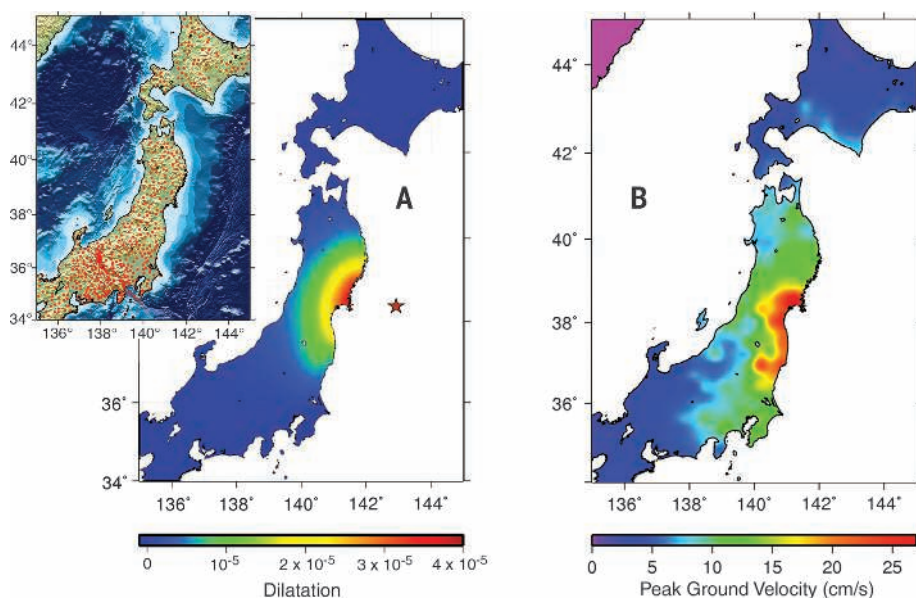


Fig. 1. Static strain and ground shaking caused by the Tohoku-Oki earthquake. (A) Modeled coseismic dilatation static strain at 5 km in depth (7). The red star shows the position of the epicenter of the Tohoku-Oki earthquake. (Inset) Positions of the Hi-net seismic stations (red points). (B) Averaged peak ground velocity measured using the KiK-net strong-motion network (7).

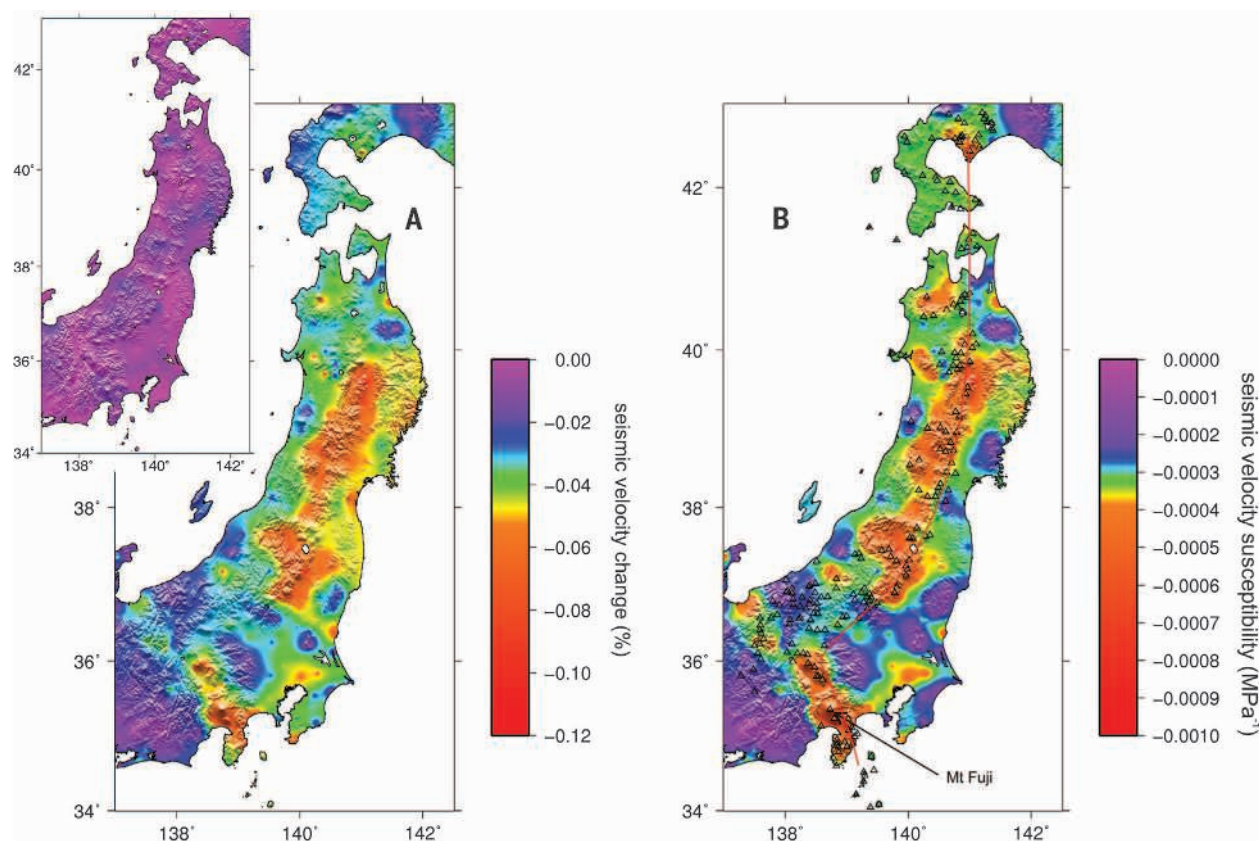


Fig. 2. Crustal seismic velocity perturbations caused by the Tohoku-Oki earthquake. (A) Coseismic crustal seismic velocity changes induced by the 2011 Tohoku-Oki earthquake. (Inset) Velocity changes averaged over 5 days preceding the day of the Tohoku-Oki earthquake. (B) Seismic velocity susceptibility computed using the seismic velocity changes shown in (A). Black triangles denote Quaternary period volcanoes, and the red line depicts the main volcanic fronts.

are known to trigger anomalous hydrothermal activity (11), aftershocks on a global scale (12), tectonic tremor activity (13), and slow-slip events (14). The origin for this remote triggering of activity is believed to be the associated dynamic stress that is caused by the passing of the seismic waves.

We used the approach of Gombert and Agnew (15) to estimate the level of dynamic strain $\Delta\epsilon$ and dynamic stress $\Delta\sigma$ from the observed peak ground velocity (PGV), such that $\Delta\epsilon \approx v/c$ and $\Delta\sigma \approx \mu v/c$, where μ is the mean crustal shear modulus ($\sim 30 \times 10^9$ Pa), v is the PGV (measured by the KiK-net, strong-motion seismograph network installed in boreholes together with the Hi-net sensors), and c is the mean wave phase velocity of the Rayleigh waves that propagate within the upper crust (~ 3 km/s). The dynamic strain caused by the passing of the seismic waves was one to two orders of magnitude higher than the static coseismic strain for Honshu Island. We thus conclude that the dynamic stress associated with the seismic waves emitted by the Tohoku-Oki earthquake was the main cause of the large seismic velocity reductions under the volcanic regions—in particular, the Mt. Fuji area, where the static stress change can be considered negligible. We then defined the seismic velocity susceptibility as the ratio between the observed reductions in the seismic velocity and the estimated dynamic stress. The distribution of these seismic velocity susceptibilities correlates with the main volcanic areas (Fig. 2B).

The sensitivity of the seismic velocity to stress changes in the rock increases with decreasing effective pressure (16, 17). Under volcanic areas, the effective pressure in the crust can be reduced because of the presence of highly pressurized hydrothermal and magmatic volcanic fluids at depth. We thus argue that the observed strong coseismic velocity reductions delineate the regions where such pressurized volcanic fluids are present in the upper crust. An important implication of our observation is that the seismic velocity susceptibility to stress can be used as a proxy to the level of pressurization of the hydrothermal and/or magmatic fluids in volcanic areas. So far, this susceptibility is greatest in the Mt. Fuji area and along the Tohoku volcanic arc, where it reached 15×10^{-4} MPa $^{-1}$, whereas it is more than 10 times smaller for the Cretaceous stiff plutonic regions of eastern Tohoku (Fig. 2B).

Fluids are also known to have important roles in earthquake nucleation (3). The volcanic areas where large seismic velocity susceptibility was observed were also characterized by large triggered seismic activity after the Tohoku-Oki earthquake (18, 19), including a particularly strong (magnitude 6.4) earthquake that occurred 4 days after the main shock, near Mt. Fuji. This confirms that the crust in these areas is quite sensitive to strong transient stress perturbations. We argue that mapping the susceptibility of seismic velocities to dynamic stress changes can be used to image and characterize regions with low effective pressure, such as volcanic systems.

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NEURODEVELOPMENT

Parasympathetic neurons originate from nerve-associated peripheral glial progenitors

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The peripheral autonomic nervous system reaches far throughout the body and includes neurons of diverse functions, such as sympathetic and parasympathetic. We show that the parasympathetic system in mice—including trunk ganglia and the cranial ciliary, pterygopalatine, lingual, submandibular, and otic ganglia—arise from glial cells in nerves, not neural crest cells. The parasympathetic fate is induced in nerve-associated Schwann cell precursors at distal peripheral sites. We used multicolor Cre-reporter lineage tracing to show that most of these neurons arise from bi-potent progenitors that generate both glia and neurons. This nerve origin places cellular elements for generating parasympathetic neurons in diverse tissues and organs, which may enable wiring of the developing parasympathetic nervous system.

Almost all bodily functions are regulated by the autonomic nervous system. Most autonomic neurons arise from migrating neural crest cells, at least in the chick (1, 2). However, unlike the neural crest-derived sympathetic, sensory, and enteric nervous systems, for which the migratory paths and cellular origins have been studied in detail, little is known about the developmental origin of the parasympathetic nervous system. Available data have been difficult to reconcile in the mouse because migration of the cranial neural crest ceases around embryonic day 10 (E10), although cranial parasympathetic ganglia do not coalesce until around E12 (3, 4). Schwann cell precursors associated with nerves produce myelinating and non-myelinating Schwann cells, endoneurial fibroblasts,

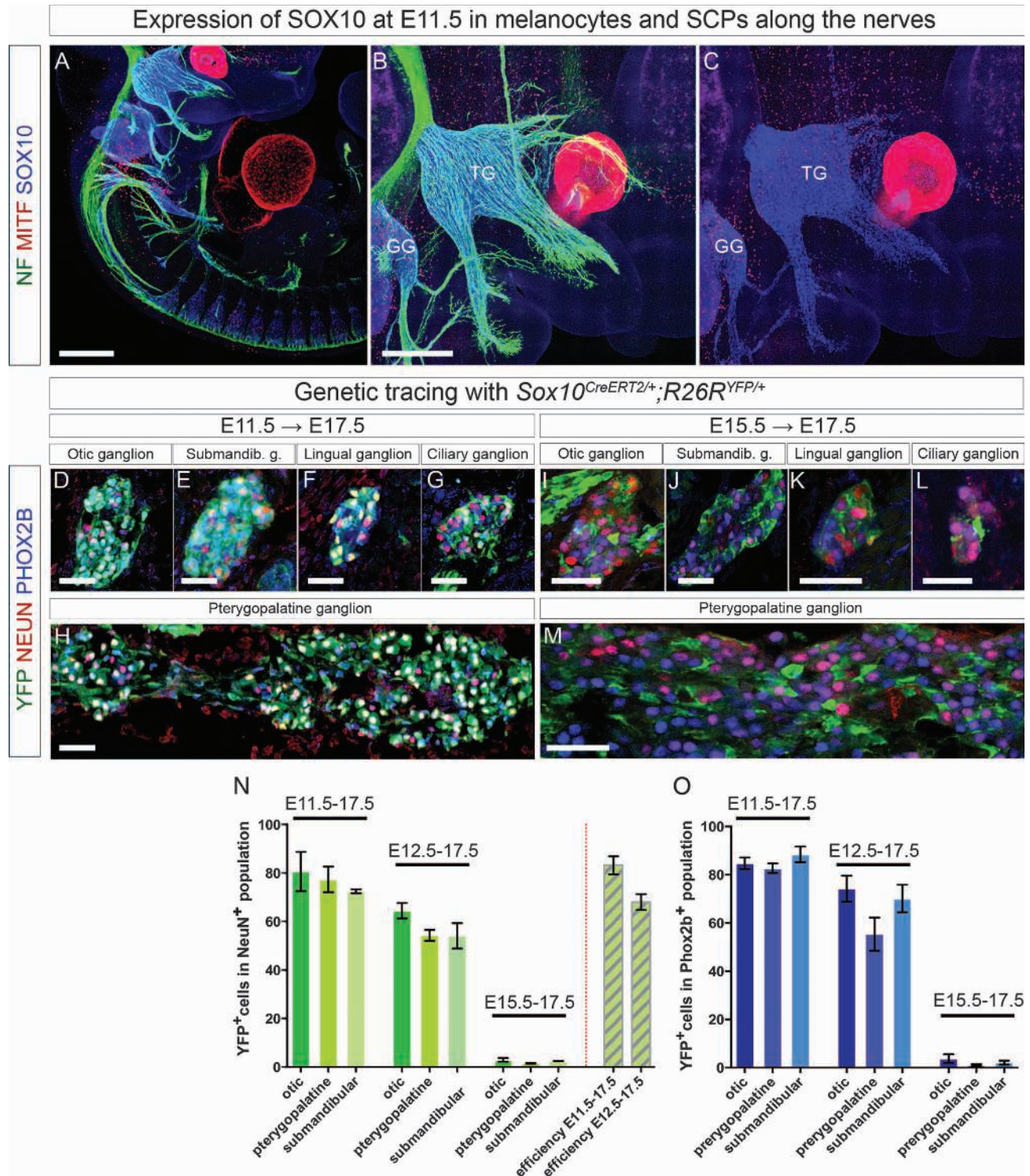
and melanocytes during development (5) and can therefore be considered as multipotent stem-like cells. The role of Schwann cell precursors

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during parasympathetic development has not been addressed. We sought to understand how the cranial parasympathetic nervous system is

built around the nerves that later in development are known to course through or serve the ganglia.

We used three-dimensional (3D) reconstruction of whole mouse embryos to address whether neural crest migration has ceased before formation



of the parasympathetic ganglia. The SRY-related high-mobility-group domain transcription factor (SOX10) labels neural crest cells (NCCs), Schwann cell precursors, and melanocytes (6, 7). Our results demonstrated that SOX10⁺ cells were either nerve-associated or SOX10⁺;MITF⁺ melanoblasts at E11.5 (Fig. 1, A to C), confirming that NCC migration has ceased at E11.5.

In chick, with the possible exception of a small number of cranial ciliary ganglion neurons (2), both glial cells and neurons of the autonomic system are of neural crest origin (8), and SOX10 is rapidly down-regulated during neurogenesis. We performed inducible tissue-specific genetic tracing to study the origin of parasympathetic neurons from SOX10⁺ progenitor cells in mouse. Transgenic mice expressing the improved tamoxifen-inducible Cre recombinase (CreERT2) under the *Sox10* promoter were crossed to the *Rosa26R^{YFP}* reporter mouse strain that contains a floxed stop cassette preventing expression of yellow fluorescent protein (YFP). Activation of genetic recombination by tamoxifen injection at E11.5–E12.5 resulted in a majority of para-

sympathetic neurons being fluorescent at the analysis stage (E17.5), which also being paralleled by the analysis of recombination efficiency, confirmed that parasympathetic neurons entirely originate from SOX10⁺ cells allocated to the nerve sites (Fig. 1, D to H, N, and O; and fig. S1). We observed a near complete lack of contribution from SOX10⁺ cells to parasympathetic neurons (only 2.29 ± 0.97% neurons were YFP, *n* = 6) in experiment when tamoxifen was administered at E15.5 (Fig. 1, I to M, N, and O). Similar results were obtained when analyzing trunk pelvic ganglia associated with splanchnic parasympathetic efferent nerve and parasympathetic ganglia of the heart (fig. S2). The association of SOX10⁺ cells with the nerves at E11.5–E12.5 (Fig. 1, A to C) suggests a nerve-dependent Schwann cell precursor origin of parasympathetic neurons.

The proneural basic helix-loop-helix (bHLH) transcription factor achaete-scute complex homolog 1 (ASCL1) and paired-like homeobox 2b (PHOX2B) homeodomain transcription factor are expressed in parasympathetic ganglia and are essential for their development (9–12). ASCL1

is believed to define a commitment of parasympathetic neurons. We crossed *Ascl1^{CreERT2}* mice to *Rosa26R^{Tomato}* reporter strain for genetic cell lineage tracing. 3D visualization revealed an absence of ASCL1^{TOMATO+} parasympathetic progenitors at E11.0 (24 hours tracing) (Fig. 2, A to D) but presence at E11.5 (24 hours tracing) (Fig. 2, E and F). These first parasympathetic neurons were invariably identified at the greater superficial petrosal nerve (gspn, a branch of facial nerve VII, 2.0 ± 0.29% cells, *n* = 3) and also at a nervous plexus formed by the oculomotor nerve (cranial nerve III, 21.83 ± 0.44% cells, *n* = 3) and ophthalmic branch of the trigeminal nerve (V1, sensory nerve) (Fig. 2, E and F). At E11.5, ASCL1^{TOMATO+} cells were not found in the Jacobson's nerve (jn, tympanic nerve) and at the chorda tympani (ct) nerves (Fig. 2, G and H). Oculomotor nerve, gspn, ct, and jn carry the preganglionic parasympathetic motor efferent (visceromotor) fibers for the ciliary, pterygopalatine, submandibular, lingual (intramuscular ganglia of the tongue), and otic ganglia. At E12.5, ASCL1^{TOMATO+} cells were observed in association with chorda tympani nerve

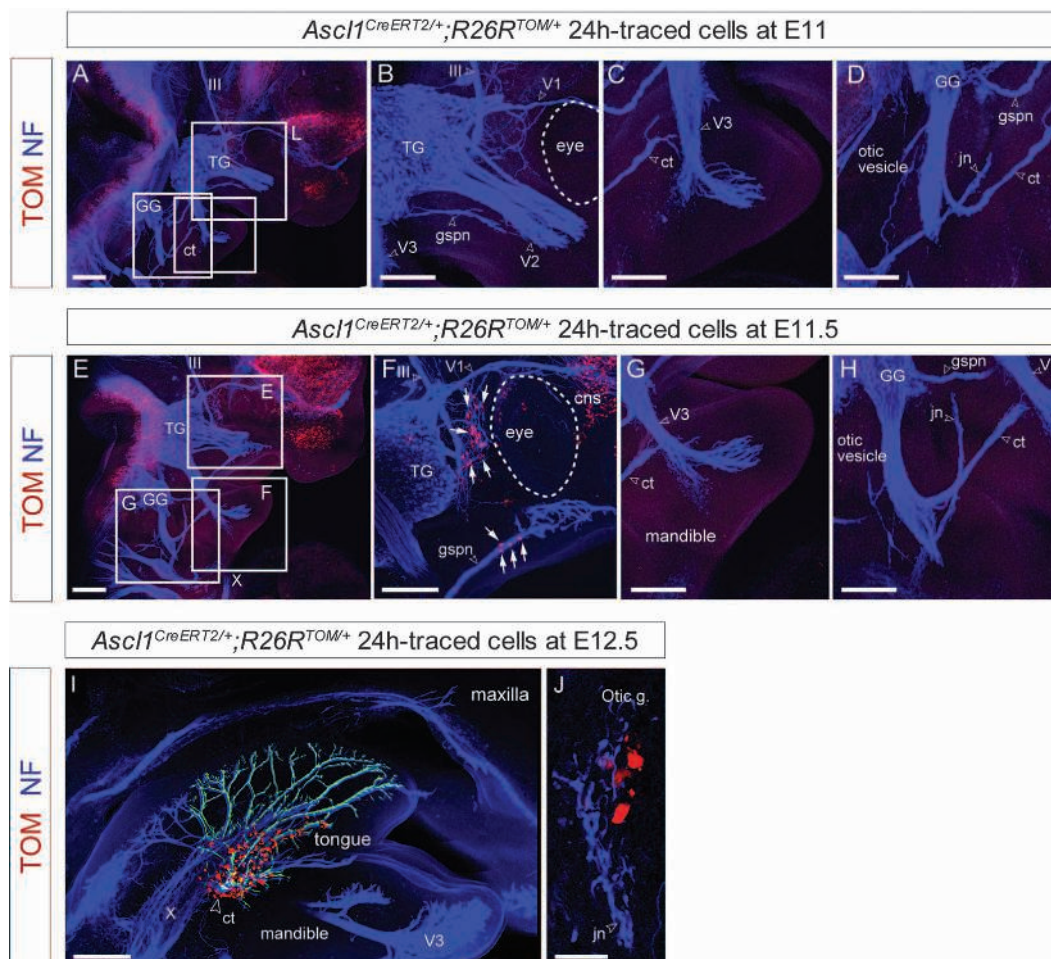


Fig. 2. Ascl1-expressing parasympathetic progenitors arise within nerves between E11.5 and E12.5. (A to J) Whole-mount staining for TOMATO and neurofilaments (NF) on 24-hours-traced *Ascl1^{CreERT2/+};R26R^{Tomato/+}* embryos. Green indicates an artificial outline of main ct fibers. TG, trigeminal ganglion; GG, geniculate ganglion; ct, chorda tympani; III, oculomotor cranial nerve; X, vagal nerve; V1, ophthalmic branch of trigeminal nerve; V2, maxillary branch of trigeminal nerve; V3, mandibular branch of trigeminal nerve; gspn, greater superficial petrosal nerve; jn, Jacobson's nerve. Roman digits reflect corresponding cranial ganglia and nerves. Scale bars, 150 μm (A to H); 100 μm (I); and 50 μm (J).

in the mandible and Jacobson's nerve (Fig. 2, I and J). The late emergence of $ASCL1^{TOMATO+}$ cells in chorda tympani coincided with later development of the tongue primordium. A nerve dependence was directly confirmed in the glial cell-line-derived family of ligands receptor *Ret*-null mutant mice (*Ret*^{CFP/CFP} mice). These mice specifically lacked the entire gspn and jn, whereas ct and oculomotor nerves stayed intact. The selective loss of only specific nerves directly correlated with a corresponding loss of pterygopalatine and otic but not lingual (fig. S3) and ciliary ganglia (3), which are innervated by chorda tympani and oculomotor nerves. Hence, cranial parasympathetic ganglia arise from presynaptic nerves and occasionally from nerves that course through the future prospective ganglia (the sensory V1) (Fig. 2).

Consistent with the above results, $ASCL1^{TOMATO+}$ expression invariably arose from within the βIII -

tubulin-positive nerves and was initiated in cells expressing the Schwann cell precursor marker SOX10 at E11.5 (Fig. 3, A to C). Schwann cell precursor survival and proliferation rely on Neuregulin-1 ligands inserted into axons forming the nerves, which signal to Schwann cell precursors through the ERBB2 and ERBB3 receptor heterodimer complex (13). All $ASCL1^{TOMATO+}$ cells were expressing ERBB3 at E11.5 (Fig. 3, A to C), identifying these cells as Schwann cell precursors. A cell-type switch was indicated by the finding that at E12.5, $ASCL1^{TOMATO+}$ cells had markedly reduced or absent expression of ERBB3 and SOX10 (Fig. 3, D and E, gspn). In addition to $ASCL1^{TOMATO+}$ cells, PHOX2B⁺ cells were invariably associated with nerves, although more broadly along the extent of the efferent preganglionic nerves (figs. S3 and S4). PHOX2B expression level was inversely correlated with that of SOX10 and ERBB3 (Fig. 3, F to J, and fig. S4). Altogether,

these results show that nerve-associated Schwann cell precursors differentiate into $ASCL1^{+}$;PHOX2B⁺ parasympathetic neurons.

To functionally confirm a glial origin of parasympathetic neurons, we analyzed *ErbB3*^{-/-} mice, which display a depletion of Schwann cell precursors accompanying nerves at E12.5 (4, 5, 14, 15). Hence, if parasympathetic ganglia arise through a fate transition of Schwann cell precursors in nerves, *ErbB3*^{-/-} mice are expected to display deficits in neuronal numbers. Indeed, *ErbB3*^{-/-} mice had no SOX10⁺ Schwann cell precursors or parasympathetic neurons in locations of ganglia at P1 (Fig. 3, K to P).

We next examined what happens with Schwann cell precursors when they fail to convert to neurons. Because $ASCL1$ is critical for specifying the parasympathetic fate, the fate of cells that normally should have induced $ASCL1$ expression can be addressed by means of genetic lineage tracing

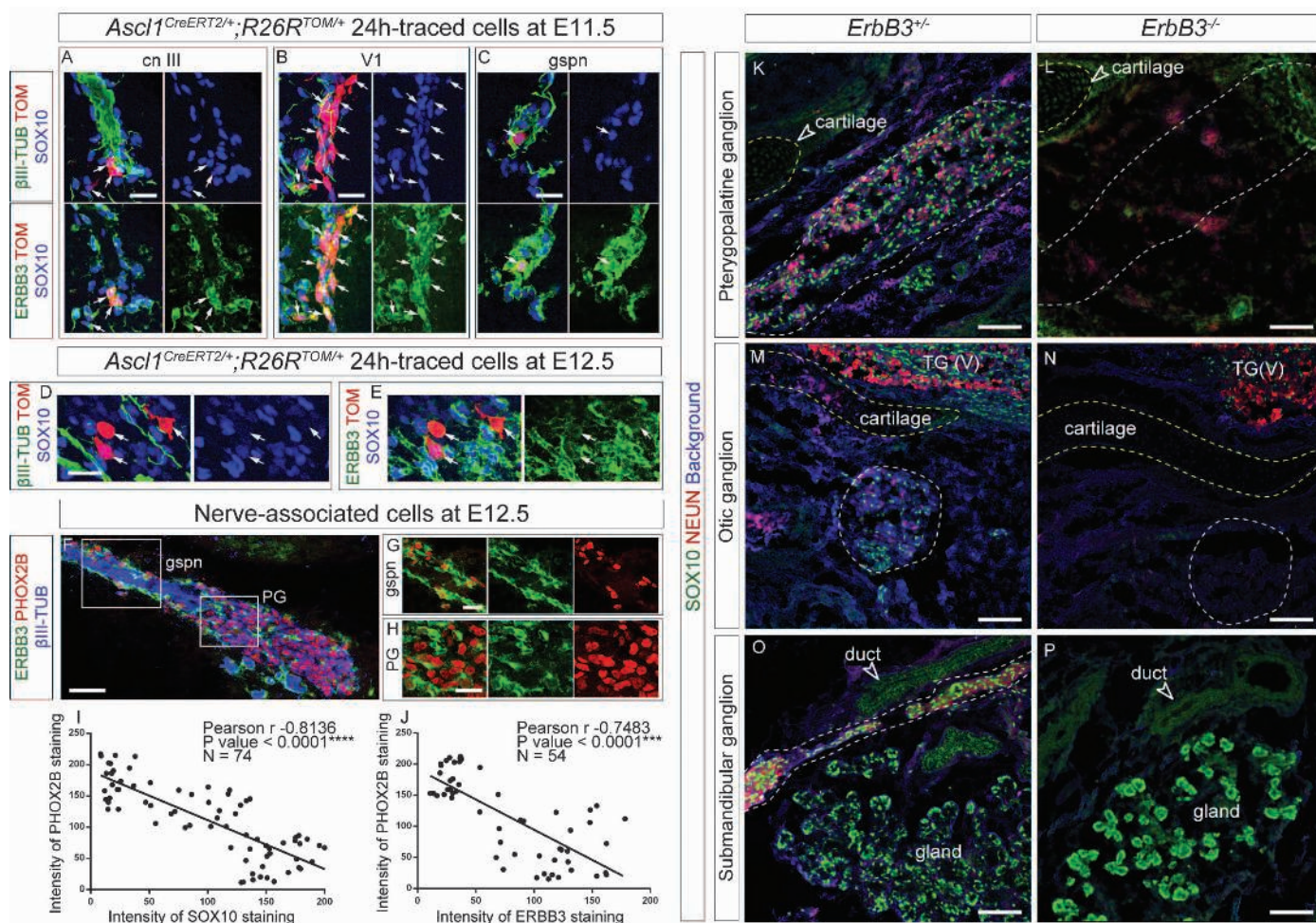


Fig. 3. Loss of Schwann cell precursor identity is associated with induction of *Ascl1* and *Phox2b*. (A to C) 24-hours-traced $ASCL1^{CreERT2/+};R26R^{TOM/+}$ embryos and SOX10⁺;ERBB3⁺;ASCL1^{TOMATO+}-traced cells in (A) cranial nerve III (cn III, arrows), (B) trigeminal nerve V1, and (C) gspn. Arrows point to $ASCL1^{TOMATO+}$;SOX10⁺;ERBB3⁺ cells. (D and E) 24-hours-traced cells in pterygopalatine ganglion of $ASCL1^{CreERT2/+};R26R^{TOM/+}$ embryos at E12.5, with variable SOX10 and ERBB3 levels. (F to H) gspn and coalescing pterygopalatine ganglion (PG) stained for PHOX2B, βIII -TUBULIN, and

ERBB3. ERBB3 is down-regulated in PHOX2B⁺ cells (H). (I and J) Correlation of (I) PHOX2B and SOX10 and (J) PHOX2B and ERBB3 expression in neurons of pterygopalatine ganglia at E12.5. (K to P) Cranial parasympathetic ganglia in [(K), (M), and (O)] *ErbB3*^{+/+} and [(L), (N), and (P)] *ErbB3*^{-/-} mice. [(K) and (L)] Pterygopalatine, [(M) and (N)] otic, and [(O) and (P)] submandibular ganglia or their expected positions are outlined by a white punctured line. Scale bars, 20 μ m (A) to (E), (G), and (H); 50 μ m (F); and 100 μ m (K) to (P).

by using *Ascl1*^{CreERT2/CreERT2};*Rosa26R*^{Tomato/+} mice (*Ascl1*-deficient mice). Lineage tracing in control *Ascl1*^{CreERT2/+};*Rosa26R*^{Tomato/+} embryos activated by tamoxifen administration at E12.5 confirmed that parasympathetic neurons are specified at this stage because neurons of lingual, otic, pterygopalatine, submandibular, and ciliary ganglia resulted to be *ASCL1*^{TOMATO+} at E17.5 (Fig. 4, A, C, E, G, and I; and fig. S5). In the absence of *Ascl1*—in *Ascl1*^{CreERT2/CreERT2};*Rosa26R*^{Tomato/+} mice—*ASCL1*^{TOMATO+}-traced cells were associated with nerves, often at anatomical locations of the missing ganglia at E17.5 (Fig. 4, B, D, F, H, and J). In the absence of *Ascl1*, *ASCL1*^{TOMATO+}-traced cells expressed the Schwann cell precursor-related markers ERBB3, SOX10, SOX2, and brain fatty acid-binding protein (BFABP; also known as FABP7), showing that in the absence of *ASCL1*, cells remained as glial cells (Fig. 4, K and L, and fig. S6). Consistently, we never observed β III-tubulin even in the remaining *ASCL1*^{TOMATO+};*PHOX2B*⁺;*SOX10*⁺

cells, which indicates a failure of neurogenesis (fig. S7, arrows). Rescuing neurogenesis through the replacement of *Ascl1* by *Neurogenin 2* (*Ngn2*) in *Ascl1*^{KINGn2/KINGn2} mice (16) was permissive for a sustained PHOX2B expression, although the progenitor cell marker SOX10 failed to be repressed (fig. S8). Hence, PHOX2B was insufficient for the repression of progenitor cell gene programs in the presence of NGN2. These results show that in the absence of a cell fate transition from glial progenitors to neurons, most of the cells that should have become parasympathetic neurons remain in nerves as glial cells.

To obtain insight on the competence of Schwann cell precursors generating parasympathetic neurons, we exploited the multicolor Cre reporter termed R26R-Confetti (17). The reporter was crossed to Proteolipid protein 1 (PLP) (*Plp*^{CreERT2}) mice. PLP is expressed in the glial compartment (5). Tamoxifen-induced recombination

resulted in expression of one out of four fluorescent proteins (Fig. 4M). Upon stochastic Cre induction by low-dose tamoxifen at E11.5 or E12.5 and collecting embryos for analysis at E17.5, individual cell clones originating from single PLP⁺ glial cells were obtained (Fig. 4, N and O). Quantification revealed a clonal relationship between neurons and glia in all parasympathetic ganglia, where recombination in individual Schwann cell precursors at E11.5 or E12.5 resulted in an average of 2.6 and 1.4 neurons at E17.5, respectively (Fig. 4P). The percentages of neurons in mixed lineage clones were 38 and 30% when traced from E11.5 and E12.5, respectively (Fig. 4Q). Thus, when traced from the earlier stage more neurons were generated, although the neuron/glia proportion remained unchanged. These results show that Schwann cell precursors are bi-potent, generating on the average a few neurons and two to three times more glial cells.

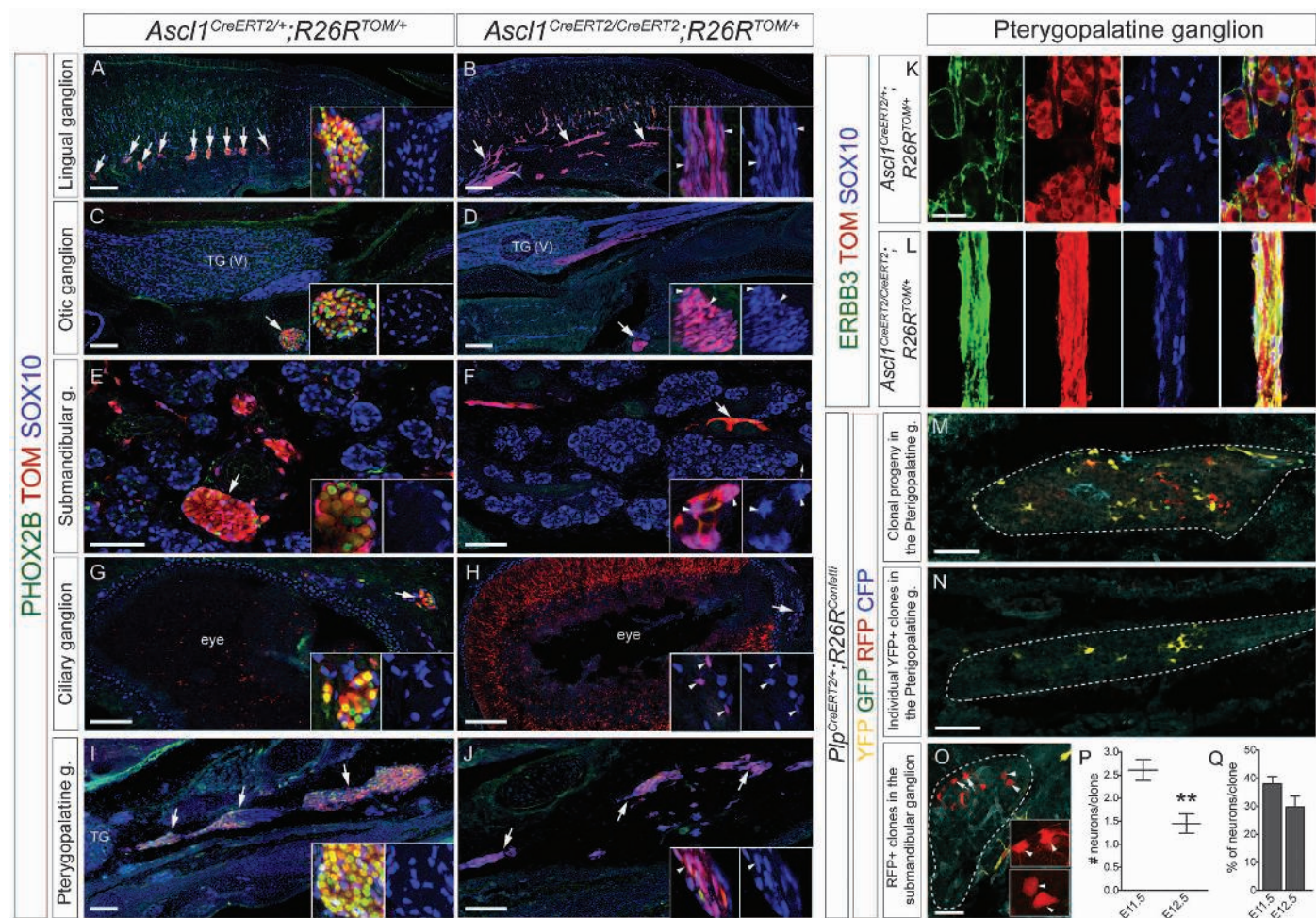


Fig. 4. Cranial parasympathetic ganglia arise from bi-potent Schwann cell precursors by induction of ACSL1. (A to J) Fate mapping of *ASCL1*-expressing cells (TOMATO) in [(A), (C), (E), (G), and (I)] control and [(B), (D), (F), (H), and (J)] *Ascl1*-deficient animals from E12.5 to E17.5. Arrows point to location of ganglia. Arrowheads point to TOMATO⁺;*SOX10*⁺ cells in *Ascl1*-deficient embryos. (K to L) Expression of glia markers ERBB3 and SOX10 in TOMATO⁺ cells from genetically traced (K) *Ascl1*^{CreERT2/+};*R26R*^{TOM/+} and (L)

Ascl1^{CreERT2/CreERT2};*R26R*^{TOM/+} embryos. TOMATO⁺ cells are co-expressing SOX10 and ERBB3 in (L). (M to Q) Genetic color-coding of cell clones from E12.5 in *Plp*^{CreERT2/+};*R26R*^{Confetti} mice in [(M) and (N)] pterygopalatine (PG) and (O) submandibular ganglia (SG) analyzed at E17.5. Arrowheads point to neurons, and arrows point to glial cells from the same clone (O). (P) Number of neurons per clone. (Q) Percent neurons per clones. Mean and SEM, $n = 25$ individual Confetti clones. Scale bars, 100 μ m (A) to (J), (M), and (N); and 50 μ m in (K), (L), and (O).

Bone morphogenetic protein (BMP) family members act directly to induce *Ascl1* and *Phox2b* (*12*), both of which are required for induction of the parasympathetic neuron fate (*18, 19*). Thus, induction of a neuronal fate driven by rising BMP levels in nerves at sites of neuronal induction could explain the derivation of neurons of the parasympathetic nervous system from progenitors that otherwise give rise to Schwann cells. This interpretation is consistent with the observation that BMP7 is expressed at the site of ciliary ganglion formation (*12*) and that it has been implicated in induction of parasympathetic neurons from sciatic-nerve NCCs (*20*). In contrast to broad expression of PHOX2B along the nerves associated with parasympathetic ganglia, ASCL1⁺ cells are restricted to the distal nerve sites at the future coalescing ganglia. Consistently, genetic tracing confirms that ASCL1 is intimately associated with a neuronal fate. Thus, our data describe the origin of the parasympathetic system, in which its neurons are recruited from glial progenitors dwelling in cranial and trunk nerves by a local induction of ASCL1 (fig. S9). The parasympathetic nervous system develops later than the sensory and sympathetic nervous systems and is located closer to the target tissues. Our results explain how derivation of postsynaptic neurons from nerves solves the problem of building nervous ganglia in distal peripheral tissues and organs during postneural crest developmental stages.

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materials transfer agreements (MTAs) of future users: *Sox10^{CreERT2}* and *Ascl1^{NG2}* (MTA from MRC), *ErbB3* KO (MTA from the Max Delbrück Center for Molecular Medicine), and *Ret^{CFP}* (MTA from RIKEN). MTA for *Ret^{CFP}* mice is attached with submission. The supplementary materials contain additional data.

SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S9
References (21–25)

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NEURODEVELOPMENT

Parasympathetic ganglia derive from Schwann cell precursors

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Neural crest cells migrate extensively and give rise to most of the peripheral nervous system, including sympathetic, parasympathetic, enteric, and dorsal root ganglia. We studied how parasympathetic ganglia form close to visceral organs and what their precursors are. We find that many cranial nerve-associated crest cells coexpress the pan-autonomic determinant *Paired-like homeodomain 2b* (*Phox2b*) together with markers of Schwann cell precursors. Some give rise to Schwann cells after down-regulation of PHOX2b. Others form parasympathetic ganglia after being guided to the site of ganglion formation by the nerves that carry preganglionic fibers, a parsimonious way of wiring the pathway. Thus, cranial Schwann cell precursors are the source of parasympathetic neurons during normal development.

Parasympathetic ganglia form one of the three divisions of the peripheral autonomic nervous system. They are located deep in cephalic, thoracic, and abdominal tissues, close to or embedded in their target organs, on which they exert actions opposite to sympathetic ganglia. The systems together are essential for cardiovascular, respiratory, and digestive functions (*1*). Activity of parasympathetic neurons is modulated by preganglionic, or visceromotor, neurons, most of which are located in the hindbrain and project in cranial nerves (*2*). Like all other peripheral autonomic neurons, parasympathetic neurons derive from the neural crest (*3*). Migration pathways of sympathetic and enteric precursors toward the dorsal aorta or in the walls of the gut are well charted (*4*). We studied how parasympathetic precursors are dispatched in the embryo to arrive at sites far away from their origin. Genetic deletion of two branches of the facial nerve (nVII) eliminated their associated parasympathetic ganglia, suggesting a role for cranial nerves in parasympathetic gangliogenesis (*5*). We thus sought

to analyze the role of cranial nerves in the development of other parasympathetic ganglia: the otic ganglion, which innervates the parotid gland, and the cardiac ganglia, which innervate the heart (*6*).

The proneural gene *Neurog2* is expressed during the differentiation of viscerosensory neurons in the geniculate (gVII), petrosal (gIX), and nodose (gX) ganglia that project in the facial (nVII), glossopharyngeal (nIX), and vagus (nX) nerves, respectively (*7*). In *Neurog2^{CreERT2/CreERT2}* embryos [hereafter *Neurog2* knockout (KO) (*8*)], cranial ganglia and nerves were affected to various extents: gVII was atrophic, and nVII reduced to its main branch (*5*) (Fig. 1, A and C and B and D); gIX was absent and so was nIX and its branch, Jacobson's nerve (*7*) (Fig. 1, A and C and fig. S1); gX was merely atrophic (fig. S1), and nX was preserved (Fig. 1, A and C). Subsequently, the otic ganglion, which normally forms at the end of Jacobson's nerve, was absent, whereas the cardiac ganglia, which normally lie on cardiac branches of nX, were present (Fig. 1, B and D). *Neurog2* is never expressed in the otic or any other parasympathetic ganglion (fig. S2). Thus, deletion of the otic ganglion in *Neurog2* KO is non-cell-autonomous and most likely results from the deletion of Jacobson's nerve. To further test whether the cardiac ganglia depend on nX, we examined embryos lacking both *Neurog2* and its paralog *Neurog1*. In *Neurog1/Neurog2* double KO, gX was absent (fig. S3), but nX still formed (Fig. 1, A and E)

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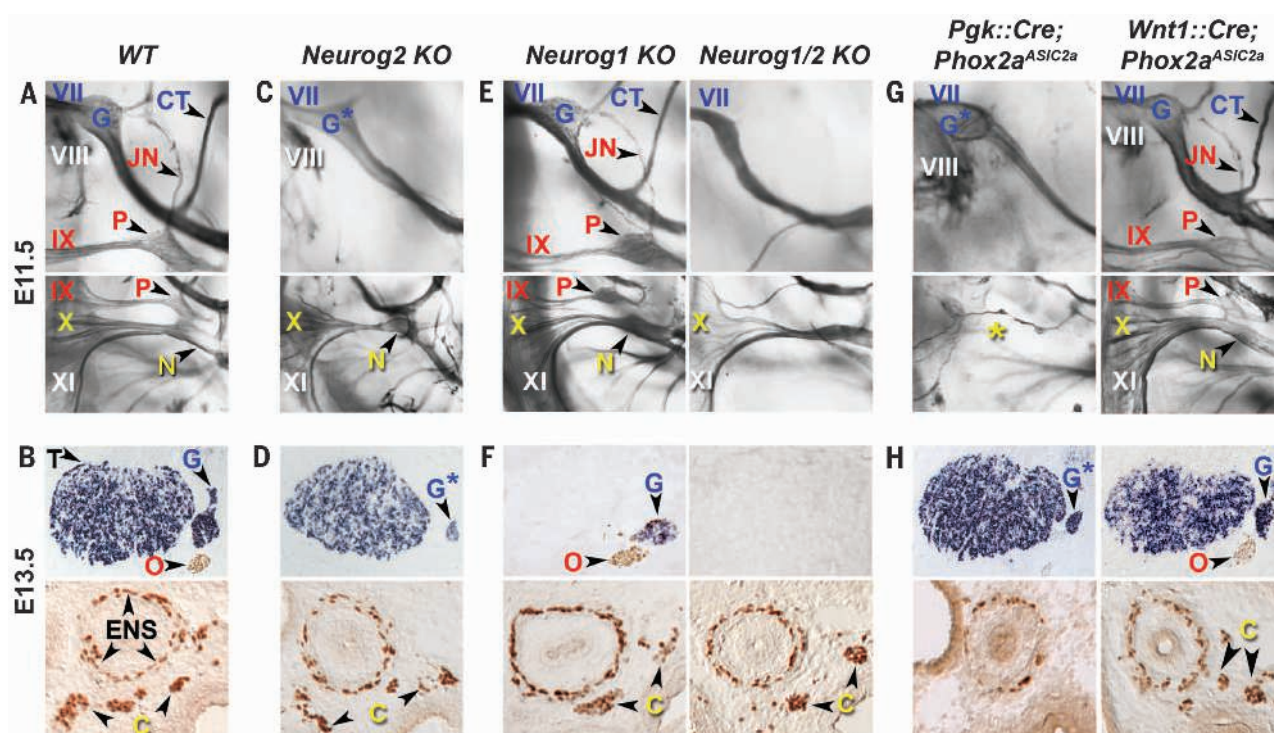
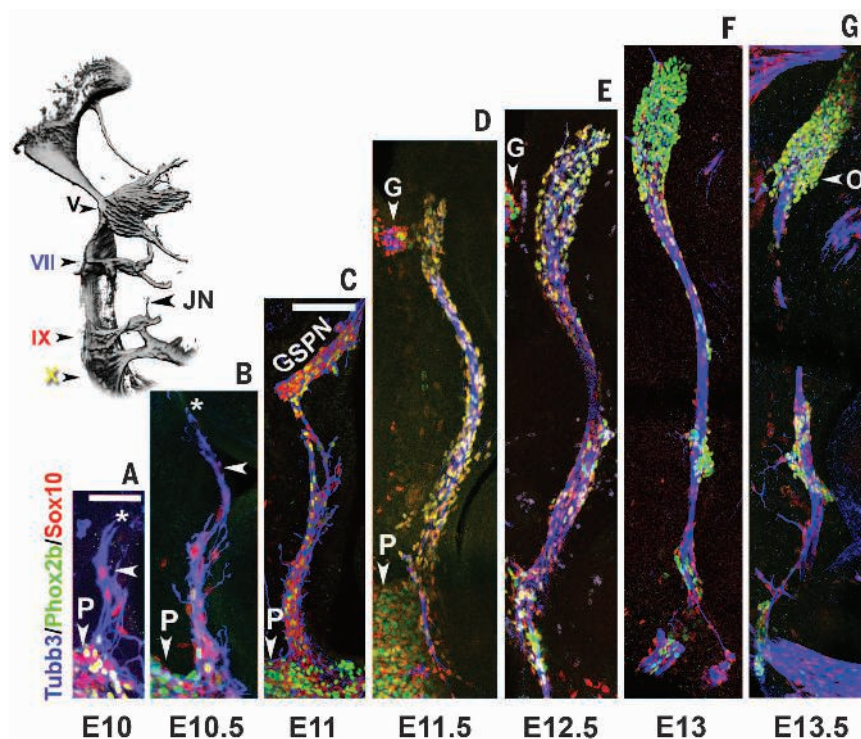


Fig. 1. Dependency of parasympathetic ganglia on cranial nerves. (A, C, E, and G) Views of whole-mount embryos at E11.5 stained for neurofilament and centered on cranial nerves VII, VIII, and IX (top) or IX, X, and XI (bottom) in wild-type (WT) embryos (A) and the indicated genotypes (C, E, and G). Dorsal is left, rostral up. (B, D, F, H) Immunohistochemistry for PHOX2b combined with in situ hybridization for *peripherin* at E13.5 on parasagittal sections through the head (top) or transverse sections through the thorax

(bottom) in WT embryos (B) and the indicated genotypes (D, F, and H). In *Neurog1* KO, only the somatosensory neurons of the vestibulo-acoustic (VIII) and trigeminal (T) ganglia are missing, with no incidence on parasympathetic ganglia. C, cardiac ganglia; CT, chorda tympani; ENS, enteric nervous system; G, gVII (geniculate ganglion); G*, atrophic gVII (corresponding to its somatic part); JN, Jacobson's nerve; N, gX (nodose ganglion); O, otic ganglion; P, gIX (petrosal ganglion); yellow asterisk, vestigial nX.

Fig. 2. Stages in the formation of the otic ganglion.

(A to G) Detection of TUBB3, SOX10, and PHOX2b on Jacobson's nerve (JN) on the three-dimensional rendering of a whole-mount neurofilament stain of cranial nerves V, VII, IX, and X at E10.5 in the top left) and the otic ganglion that forms at its distal end. (A and B) The most distal SOX10⁺ cell (arrowheads) lags behind the most advanced fibers (asterisks). (E to G) A cluster of PHOX2b⁺/SOX10^{weak} cells forms a third of the way along JN, presumably corresponding to a previously undescribed parasympathetic ganglion. GSPN, greater superficial petrosal nerve; other abbreviations as in Fig. 1. Scale bars indicate 50 μ m in (A) and (B) and 100 μ m for (C) to (G).



(made solely of motor fibers) and so did the cardiac ganglia (Fig. 1, B and F). We thus attempted to destroy nX by deleting motor in addition to sensory fibers. Having observed that Cre-dependent

diphtheria toxin chain A action (9) was too slow for the required time frame, we adapted a technique originally developed in *Caenorhabditis elegans* (10), conditionally expressing a toxic allele of the sodium

channel ASIC2a (11) under the promoter of *Paired-like homeodomain 2a* (*Phox2a*) (fig. S4). In *Pgk::Cre; Phox2a^{ASIC2a}* embryos (where *Cre* is expressed in the germ line), precursors of viscerosensory neurons should be killed in the epibranchial placodes and visceromotor neurons upon exit of the cell cycle, according to the expression schedule of *Phox2a* (12). As predicted, both types of neurons were absent in embryonic day 11.5 (E11.5) *Pgk::Cre; Phox2a^{ASIC2a}* pups (fig. S5). The main branch of nVII persisted (Fig. 1, A and G), most likely formed by the projections of somatosensory neurons that are located in the proximal part of gVII and do not express *Phox2* genes (13); nIX and Jacobson's nerve were missing (Fig. 1, A and G) and so was the otic ganglion (Fig. 1, B and H). As to nX, it was reduced to a vestigial ramus (Fig. 1, A and G), and cardiac ganglia failed to form (Fig. 1, B and H). The agenesis of parasympathetic ganglia in *Pgk::Cre; Phox2a^{ASIC2a}* embryos was non-cell-autonomous: When we recombined the *Phox2a^{ASIC2a}* locus selectively in premigratory neural crest with a *Wnt1::Cre* allele (14)—thus, in sympathetic and parasympathetic precursors—sympathetic ganglia were absent at E13.5 (fig. S6), but parasympathetic ganglia were unaffected (Fig. 1, B and H). They degenerated only one day later (fig. S6), in line with the fact that they start expressing *Phox2a* at E12.5, that is, 2 days later than sympathetic ganglia (15). Therefore, the absence of cardiac ganglia in *Pgk::Cre; Phox2a^{Stop/ASIC2a}* at E13.5 is most likely secondary to the atrophy of nX. In summary, the *Neurog2* KO and *Pgk::Cre; Phox2a^{ASIC2a}* mutations each delete a different set of cranial nerves cell-autonomously, and the corresponding parasympathetic ganglia fail to form. We conclude that parasympathetic ganglia formation depends on the cranial nerves that innervate them.

This dependency suggests that the nerves form before the ganglia and then recruit the ganglionic cells. To gather evidence for this, we monitored the formation of Jacobson's nerve and the behavior of neural crest cells in its vicinity by combined detection of β III-tubulin (TUBB3), SOX10 [an early marker for neural derivatives of the neural crest (16)], and PHOX2b [a general determinant of autonomic ganglia (17)]. Between E10 and E11 (Fig. 2, A to C), in short sequence, Jacobson's nerve emerged from the distal pole of gIX and projected toward its endpoint, close to gVII, accompanied by SOX10⁺ cells with elongated nuclei, which lagged behind the most advanced fibers (Fig. 2, A and B) and whose numbers progressively increased. The pan-autonomic determinant *Phox2b* was switched on along the nerve in a salt-and-pepper manner between E10.5 and E11 (Fig. 2, B and C) and was expressed by all SOX10⁺ nerve-associated cells at E11.5 (Fig. 2D). Between E12.5 and E13.5, the otic ganglion anlage, first visible as a buildup of cells around the distal end of the nerve (Fig. 2E), condensed and became demarcated from the nerve, which was concomitantly depleted of cells (Fig. 2, F and G). At the same time, *Phox2b* and *Sox10* expression domains became mutually exclusive, PHOX2b weak or absent in most nerve-associated cells, and SOX10 weak in most PHOX2b⁺ ganglion cells. Nerve-associated

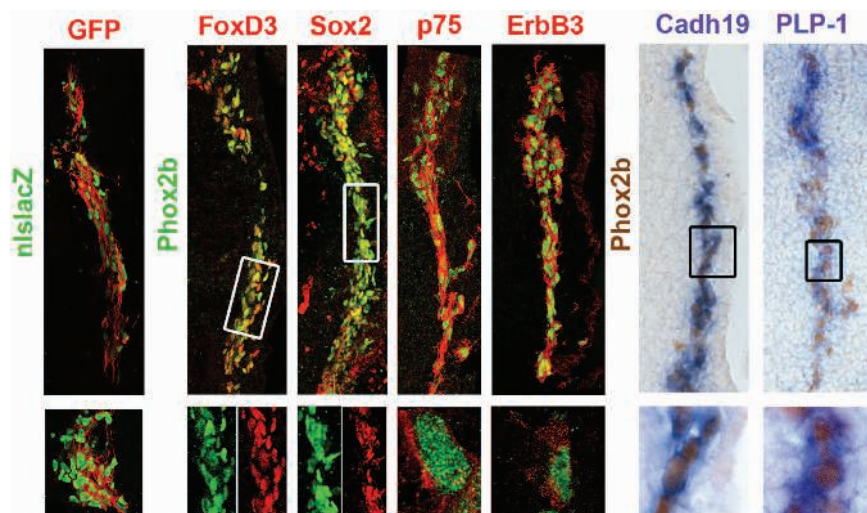


Fig. 3. Molecular signature of parasympathetic precursors. Labeling of Jacobson's nerve and associated cells at E11.5 with the indicated antibodies and probes. (Top left) Intersectional lineage tracing for a history of *Phox2b* expression and a neural crest origin in a *Phox2b::FLPo; Wnt1::Cre; RC::Fela* embryo. Green fluorescent protein (GFP) and a nuclear-localized *lacZ* (*nls lacZ*) are expressed from the *RC::Fela* allele (21), respectively, after the action of *FLPo* recombinase under the control of *Phox2b* (20) or after the combined actions of *Phox2b::FLPo* and *Cre* under the promoter of *Wnt1* (14). The fibers that belong to viscerosensory and visceromotor neurons (PHOX2b⁺ but not derived from the neural crest) are GFP⁺ (red), whereas the cells along the nerve (PHOX2b⁺ and derived from the neural crest) are *nls lacZ*⁺ (green). (Bottom left) Anlage of the otic ganglion at E11.5. All other bottom images are enlargements of a boxed area or a single cell from the top images.

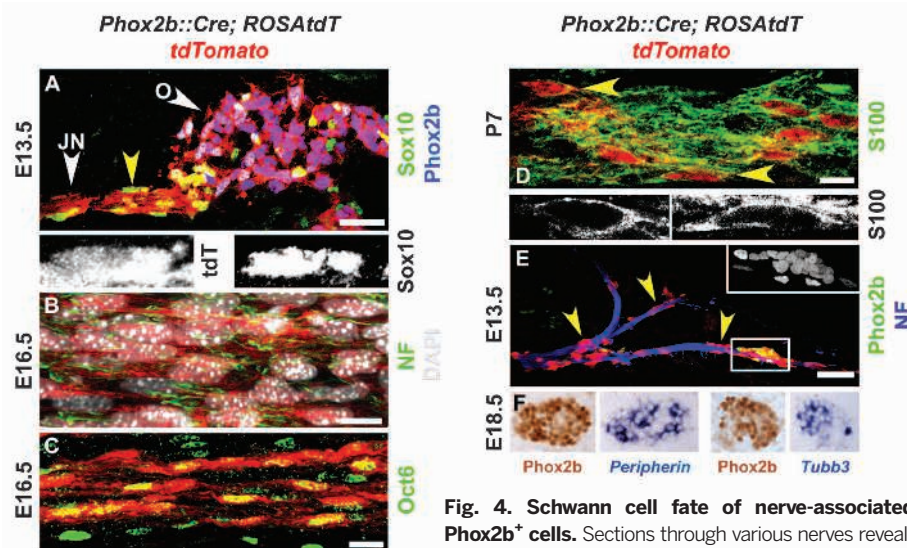


Fig. 4. Schwann cell fate of nerve-associated *Phox2b*⁺ cells. Sections through various nerves revealing tdTomato (tdT) fluorescence triggered by a *Phox2b::*

Cre and stained by DAPI (4',6-diamidino-2-phenylindole) and antibodies as indicated. (A) SOX10⁺ cells associated with Jacobson's nerve (yellow arrowhead) are tdT⁺, hence have expressed *Phox2b* but no longer do, whereas cells in the otic ganglion (O) have maintained PHOX2b expression; bottom images are enlargements of the cell marked in the top image. (B) Segment of nX immunostained for neurofilament (NF) at E16.5. (C) Section through a lingual nerve at E16.5. (D) Section of the greater superficial petrosal nerve at P7. Bottom images are the S100 signal in the cells marked in the top image. (E) Section through the median nerve of the upper limb at E13.5 showing cells with a history of *Phox2b* expression along the nerve and a cluster of *Phox2b*⁺ cells (inset, close up of anti-PHOX2b immunofluorescence) that are neuronal, based on the detection on serial sections of *peripherin* and PHOX2b or *Tubb3* and PHOX2b (F). Scale bars are 30 μm for (A), 5 μm for (B), 10 μm for (C) and (D), and 60 μm for (E).

and ganglion cells at this stage had most likely switched off *Phox2b* or *Sox10*, respectively, because all nerve-associated cells equally expressed both genes a day earlier (and see below). The observed sequence of events suggests that the SOX10⁺ cells, which then become SOX10⁺/PHOX2b⁺ double-positive, migrate along the nerve and reach in that way the site of ganglion formation, although a directed proliferation could also play a role. Accordingly, in *Neurog2* KO, where Jacobson's nerve is absent (Fig. 1), the stream of cells was absent (fig. S1). This scenario is not unique to the otic ganglion, because a similar sequence was evident for the sphenopalatine and cardiac ganglia (fig. S7).

Schwann cell precursors, which later give rise to myelinating and nonmyelinating Schwann cells, occupy embryonic nerves from E11 (18). We therefore explored whether the nerve-associated SOX10⁺/PHOX2b⁺ cells might be Schwann cell precursors. Like Schwann cell precursors and like otic ganglion cells (fig. S8), PHOX2b⁺ Jacobson's nerve-associated cells were derived from the neural crest, as evidenced by Wnt1::Cre lineage tracing (Fig. 3). They expressed markers of neural crest (FOXD3, SOX2, and p75) and Schwann cell precursors (ErbB3, *Cadherin19*, and the myelin protein *PLP*) (Fig. 3). In contrast, none of them detectably expressed neuronal markers, such as NeuN and TUBB3, or the proneural factors *Neurog2* or *Ascl1* (fig. S9). *Ascl1* (but not *Neurog2*) was up-regulated in ganglion cells (fig. S9) in keeping with its later roles in parasympathetic ganglia (19). Thus, cranial nerve-associated parasympathetic precursors have all the hallmarks of Schwann cell precursors.

Because cranial nerve-associated cells have features of both autonomic and Schwann cell precursors, we tested whether they actually produced Schwann cells. In *Phox2b::Cre; ROSA^{tdTomato}* embryos (13), as early as E13.5, Jacobson's nerve was coated with SOX10⁺/PHOX2b⁺ cells that expressed tdTomato (Fig. 4A), confirming that PHOX2b⁺ nerve-associated cells subsequently switch it off. At E16.5, several cranial nerves contained numerous tdTomato⁺ cells intermingled with the fibers (Fig. 4B and fig. S10), most of them PHOX2b⁺, an occasional one still expressing the gene (fig. S10). A different set of recombinase and reporter [*Phox2b::FLPo* (20) and *RC::Fela* (21)] (fig. S10) produced similar results. Many cranial nerve-associated PHOX2b⁺ tdTomato⁺ cells expressed the Schwann cell markers Oct6 at E16.5 and S100 at P7 (Fig. 4, C and D) (18), signs of their differentiation into Schwann cells. Thus, cranial Schwann cells derive in part from a contingent of nerve-associated cells with a history of *Phox2b* expression. Such cells, albeit sparser, were also associated with limb nerves (Fig. 4E and fig. S11), and, from E13.5 on, we found (in 9 out of 10 limbs) a small ganglion made of PHOX2b⁺ neurons on the median nerve of the forelimb (Fig. 4, E and F), showing that the dual Schwann cell and neuron fate, although mostly associated with cranial nerves, is not unique to them. The persistence in the adult and physiological meaning, if any, of this new ganglion and the nature of its preganglionic innervation—known to control the survival of parasympathetic neurons (22)—remain to be determined.

Thus, parasympathetic ganglion neurons are derivatives of multifated Schwann cell precursors, known to give rise in vivo to melanocytes (23) and endoneurial fibroblasts (24) in addition to Schwann cells. The neuronal potential of these precursors, reported in vitro or after back transplantation for sciatic nerve-derived cells (25, 26), is achieved during the normal development of cranial nerves. Accordingly, the numerous locations of parasympathetic ganglia throughout the head and trunk are specified by the pattern of cranial nerve outgrowths, a parsimonious way of hooking up pre- and post-ganglionic partners in development and, conceivably, of synchronizing their appearance in evolution.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S11
References (27–33)

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VESSEL FORMATION

De novo formation of a distinct coronary vascular population in neonatal heart

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The postnatal coronary vessels have been viewed as developing through expansion of vessels formed during the fetal period. Using genetic lineage tracing, we found that a substantial portion of postnatal coronary vessels arise de novo in the neonatal mouse heart, rather than expanding from preexisting embryonic vasculature. Our data show that lineage conversion of neonatal endocardial cells during trabecular compaction generates a distinct compartment of the coronary circulation located within the inner half of the ventricular wall. This lineage conversion occurs within a brief period after birth and provides an efficient means of rapidly augmenting the coronary vasculature. This mechanism of postnatal coronary vascular growth provides avenues for understanding and stimulating cardiovascular regeneration following injury and disease.

Coronary artery disease causes myocardial infarction, the leading cause of death worldwide. How coronary arteries develop is a fundamental biological question with important ramifications for human health and disease (1). Defining the developmental pro-

grams that give rise to the coronary arteries will provide critical information for regenerative approaches to congenital and adult heart disease (2–4). Most previous studies of coronary developmental origins have focused on the midgestation stage, when coronary vessels initially form

over the heart (5–11). Postnatal coronary vessels were presumed to arise from these embryonic coronary vessels, but few studies have examined postnatal coronary artery growth, and this assumption has not been rigorously tested.

Embryonic coronary vessels initially form as a vascular plexus that covers the outer surface of the heart (6, 11). The underlying heart muscle is composed of compact myocardium, the free walls of the ventricular chambers, and trabecular myocardium, the fingerlike projections into the chambers (12). Compact myocardium develops intramyocardial coronary vessels, whereas trabecular myocardium largely lacks intramyocardial vessels and exchanges nutrients with the surrounding blood within the ventricular chambers. During rapid postnatal ventricular growth, the trabecular myocardium undergoes “compaction” and coalesces with compact myocardium (12). This new layer of compact myocardium becomes richly supplied with coronary vessels. The developmental program for postnatal vascularization of this newly compacted myocardium was previously unknown.

Here, we used tamoxifen-inducible Cre recombinase controlled by the apelin promoter (Apln-CreER) to permanently label embryonic vascular endothelial cells (VECs) of coronary vessels (11). A single tamoxifen injection at mouse embryonic day 10.5 (E10.5) induced recombination of Cre reporter alleles, which indelibly labeled early coronary sprouts and their progeny (11). This treatment allowed us to almost completely label coronary vessels in the embryonic ventricle wall at E15.5 (Fig. 1). We designated this coronary vascular population (CVP) labeled by Apln-CreER activation at E10.5 as the first CVP. We followed the first CVP's fate in postnatal days 0 to 7 (P0 to P7) neonatal hearts and found that labeled coronary vessels were confined to the outer half of the myocardial wall at P3 and P7 (Fig. 1). In neonatal hearts, progressive myocardial compaction was accompanied by accumulation of unlabeled coronary vessels in the inner half of the myocardial wall at P3 and P7 (Fig. 1). These unlabeled platelet endothelial cell adhesion molecule-positive (PECAM⁺) cells were bona fide coronary vessels because they were positive for the coronary VEC markers APLN (6) and fatty acid binding protein 4 (FABP4) (13) (fig. S1 to S3). These data demonstrate that the newly formed coronary vessels in the inner myocardial wall of neonatal heart are not derivatives of the first CVP, which indicates that they form de novo from an alternative source.

To get a holistic view of coronary vascular development of the entire heart, we fate-mapped coronary VECs by Apln-CreER labeling at different times during heart development. Coronary vessels emerged in three temporal waves (fig. S4). By E10.5, the first CVP had emerged in the first wave and had formed the coronary vessels of the fetal ventricular free wall (fig. S5, A and B) and postnatal outer myocardial wall (fig. S4C). However, very few VECs of the ventricular septum were labeled by E10.5 tamoxifen induction (figs. S4B and S5B). Tamoxifen administration at E13.5 identified a second wave of coronary vessel development that formed most of the robust vascular supply of the interventricular septum during septal myocardial compaction (figs. S4D and S5C) (12). Labeling of these cells by tamoxifen at E13.5, but not E10.5, therefore indicates de novo formation of these septal vessels from a source other than the first CVP. The third wave occurred in the neonatal period, and similarly to the second wave, its emergence also accompanied trabecular compaction (fig. S4G). This neonatal wave of de novo vessel formation occurred in the inner myocardial wall and remained spatially segregated from the coronary vessels formed by the first and second wave (fig. S4H).

We next sought to define the developmental origin for the de novo formed vessels that did not originate from the first CVP. We generated inducible Nfatc1-CreER;Rosa26^{RFP/+} mice (fig. S6, A and B), in which Nfatc1 regulatory elements direct selective expression of CreER to the endocardium (14). Staining of estrogen receptor (ESR) showed expression of CreER in atrial and ventricular endocardial cells in early developing heart (Fig. 2A and fig. S6, C and D). We administered tamoxifen at E8.0 to E8.5 to label E8.5 to E9.5 endocardial cells before the formation of coronary vessels (Fig. 2B) and analyzed embryos at multiple subsequent time points. Sinus venosus endocardium did not express CreER and was not labeled (see SV in Fig. 2A and fig. S7A). Nfatc1-CreER specifically labeled endocardial cells but not coronary VECs in the myocardial free wall (fig. S7, B and C). This result of inducible genetic labeling driven by Nfatc1-CreER differed from that of a previous report using constitutively active Nfatc1-Cre (see supplementary text). At P0, Nfatc1-CreER labeled 72.2% of neonatal ventricular endocardial cells and did not significantly label free wall VECs (fig. S7, D and E). In P7 hearts, 76.7 ± 5.4% of VECs in the inner myocardial wall expressed the red fluorescent protein (RFP) lineage tracer (Fig. 2C). A subset of nonlabeled VECs in the inner myocardial wall may arise from embryonic vessels or epicardium. In contrast to the inner myocardial wall, fewer VECs of the outer myocardial wall were labeled by Nfatc1-CreER (Fig. 2C). Together, these data indicate that inner myocardial wall VECs arise by lineage conversion of neonatal endocardial cells during trabecular compaction.

We also investigated the contribution of endocardial cells labeled by Nfatc1-CreER (pulse activated by tamoxifen at E8.0 to E8.5) to VECs of the

interventricular septum. In both embryonic and neonatal hearts, Nfatc1-CreER labeled the majority of coronary VECs within the interventricular septum (fig. S6E). These data indicate that the interventricular septum, like the inner myocardial wall, is vascularized by lineage conversion of endocardial cells to coronary VECs.

The inverse and nearly exclusive relation between the Apln-CreER and Nfatc1-CreER fate maps was striking (fig. S8A). This result indicates that the postnatal coronary vasculature originates from two distinct CVPs that arise through different developmental mechanisms and remain spatially segregated in their location. The first CVP, labeled by Apln-CreER induced with tamoxifen at E10.5, derives from sub-epicardial precursors that generate the initial coronary vascular plexus of the fetal hearts (6, 11) and ultimately gives rise to the VECs of the outer myocardial wall of the neonatal hearts (Fig. 1). Nfatc1-CreER labels ventricular endocardial cells, which give rise to a second CVP that ultimately irrigates the interventricular septum and VECs of the inner myocardial wall of the neonatal hearts (fig. S6E and Fig. 2C).

We next measured the relative vascular territory occupied by first and second CVPs in adult heart ventricle. Because labeling efficiency was higher when we used Apln-CreER rather than Nfatc1-CreER, we quantified with Apln-CreER labeling the relative myocardial volume irrigated by first CVP (RFP-labeled) and second CVP (unlabeled) (fig. S8, B and C). We examined serial sections spanning the entire heart at P0, P3, P7, P28, and P63 (Fig. 3A and figs. S8 and S9). At P0, the first CVP existed in 42% of the ventricular myocardium, whereas the second CVP was confined to the ventricular septum and supplied 24% of the ventricle myocardium (Fig. 3B). Trabeculae, lacking intramyocardial vessels, were still present along the luminal surface of the ventricles and accounted for the remaining 34% of the ventricular myocardium (Fig. 3B). By P7, trabecular myocardium transformed into compact myocardium, which was populated by the second CVP (Fig. 3B). In the P28 and P63 adult heart, a strong boundary was maintained at the interface of the first and second CVPs throughout the entire heart (Fig. 3A and figs. S9 and S8E). These data indicate that the vascular territory supplied by these developmentally distinct CVPs remains spatially segregated in the adult heart and that expansion of the second CVP accounts disproportionately for postnatal coronary vascular expansion (fig. S10).

To begin to understand how neonatal endocardial cells transform from a single sheet lining the heart lumen into many tube-shaped blood vessels, we observed their association with the basement membrane of trabecular myocardium during myocardial compaction. COL3A1, an extracellular matrix protein, is part of the trabecular basement membrane located just beneath the endocardial layer at P0. During subsequent myocardial compaction, COL3A1 was not degraded and left a historical mark of the remodeled trabecular protrusions (fig. S11A). Endocardial cells in the trabecular protrusions resided on the

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Fig. 1. Derivatives of embryonic coronary vessels do not expand into the inner myocardial wall of the neonatal heart. (A and B) Embryonic coronary vessels were RFP-labeled by tamoxifen treatment of *Apln-CreER;Rosa26^{RFP/+}* embryos at E10.5. In the neonatal heart, these VECs, derived from embryonic coronary vessels, occupied the outer portion of the compact myocardium, defined as the outer myocardial wall (OMW). The remaining, RFP-negative portion of the compact myocardium was defined as the inner myocardial wall (IMW). White dotted lines highlight the inner border of left ventricular myocardium occupied by embryonically labeled vessels. From P0 to P7, trabecular myocardium (Trab.) transformed into compact myocardium where new coronary vessels arise. White scale bar, 200 μ m; yellow scale bar, 50 μ m. LV, left ventricle.

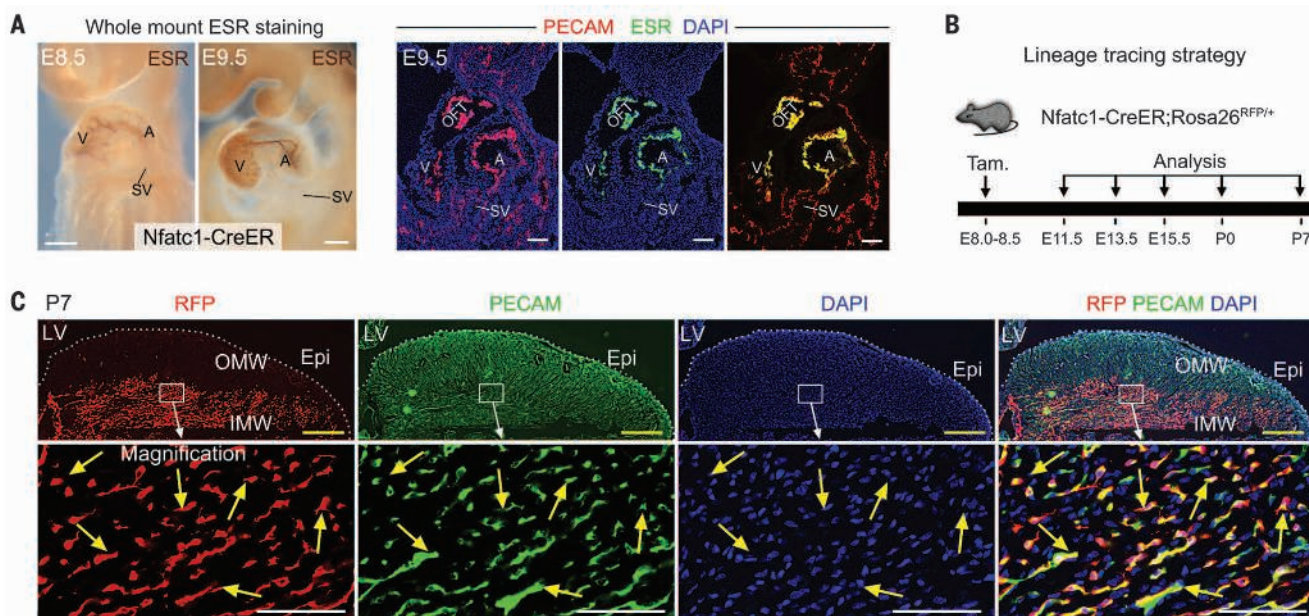
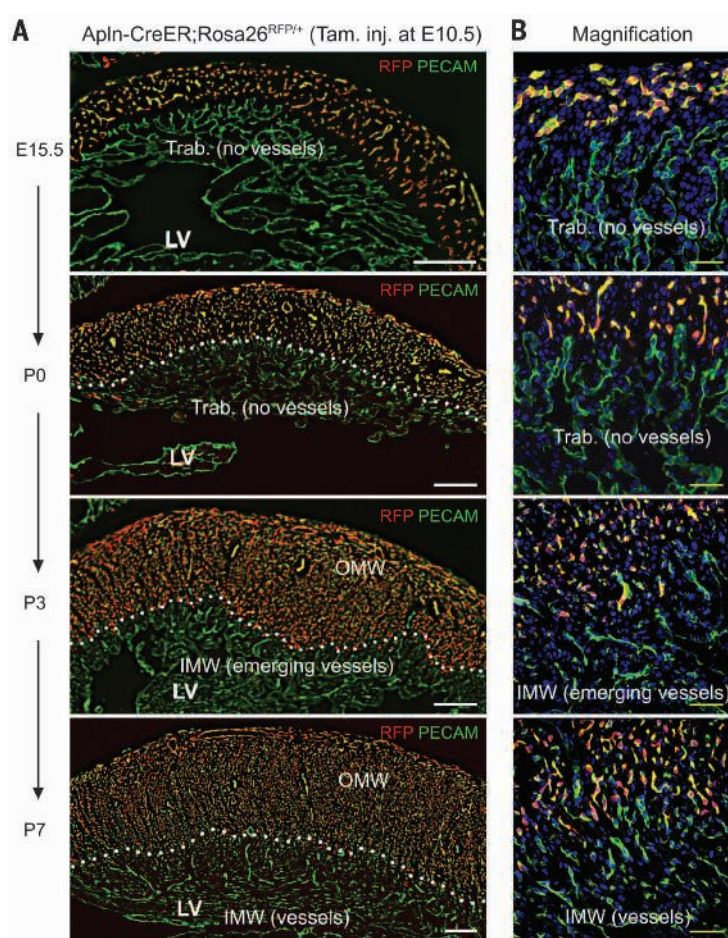


Fig. 2. Neonatal endocardial cells contribute to the de novo formed coronary vessels in the inner myocardial wall. (A) CreER was expressed in atrial and ventricular endocardium, but not sinus venosus, at E8.5 and E9.5. CreER was visualized by staining for the estrogen receptor (ESR) portion of the CreER fusion protein. V, ventricle; A, atrium; OFT, outflow tract; SV, sinus venosus. (B) Lineage tracing strategy using *Nfatc1-CreER;Rosa26^{RFP/+}* line. Tamoxifen was

injected at E8.0 to E8.5, and hearts were collected at later stages. (C) Immunostaining of genetic marker RFP and endothelial cell marker PECAM on P7 heart sections. *Nfatc1-CreER*-labeled endocardial cells contribute to the majority of VECs ($76.7 \pm 5.4\%$, yellow arrows) in the IMW, but not VECs in the OMW. White scale bar, 100 μ m, yellow scale bar, 500 μ m. $n = 3$ to 5 embryos examined at each time point.

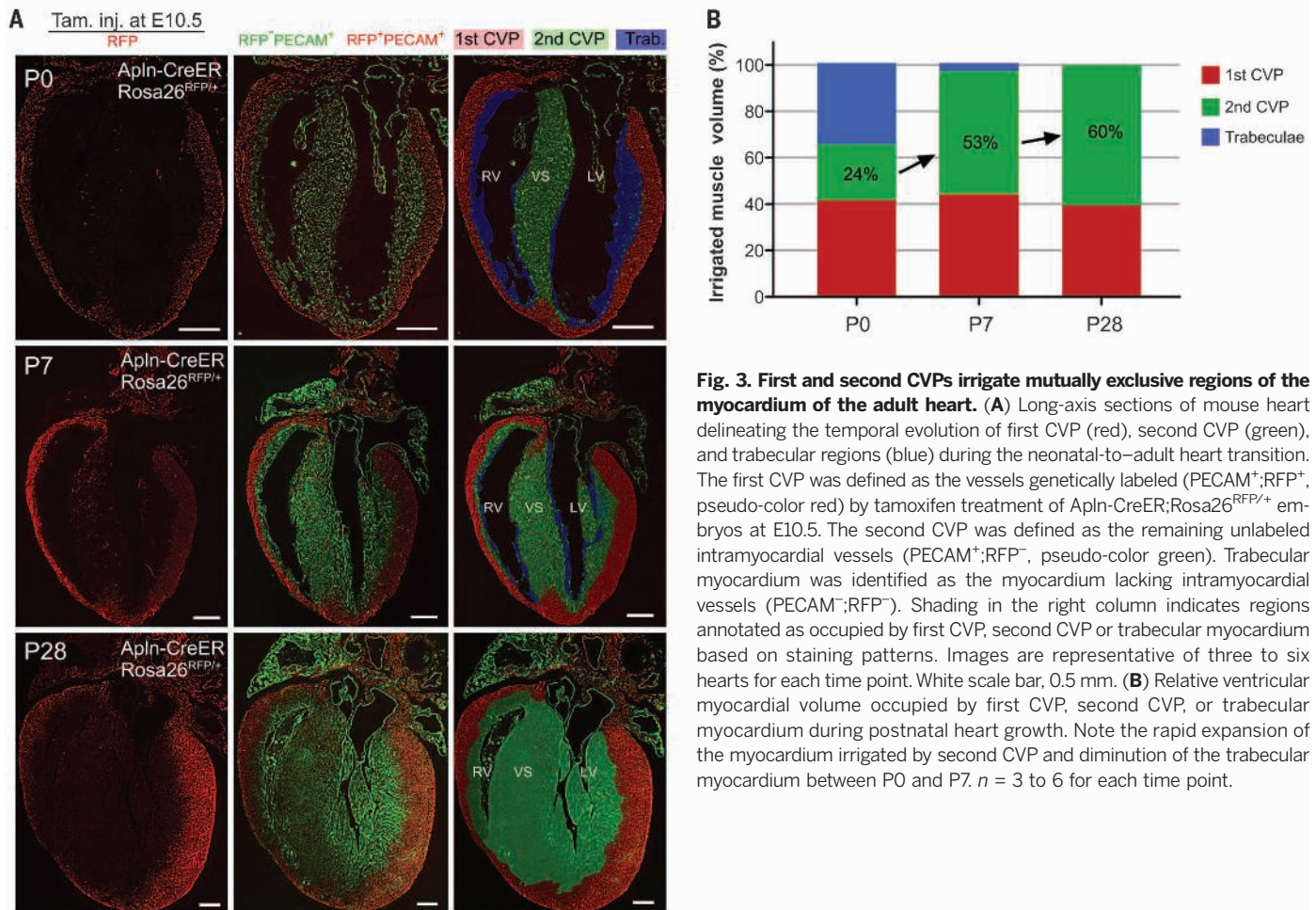
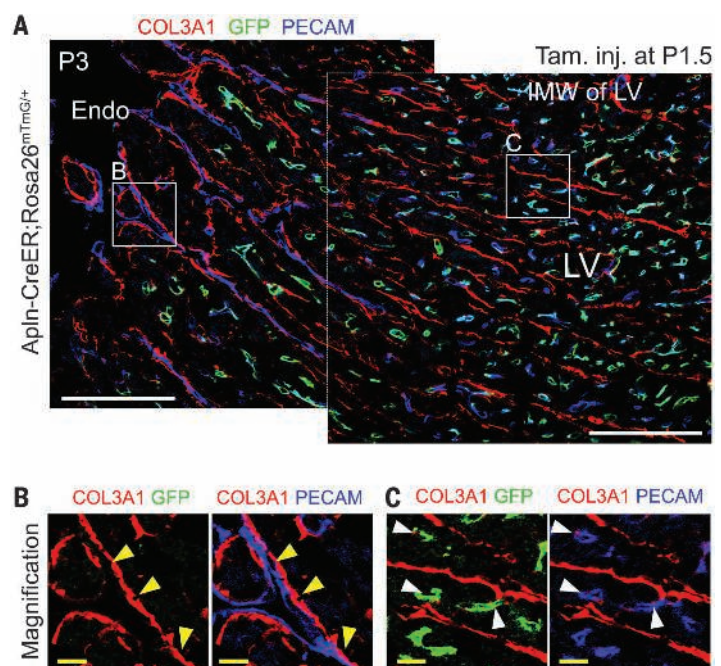


Fig. 4. Neonatal endocardial cells were trapped in the inner myocardial wall. (A) Coronary vessels forming in the myocardial wall were labeled by tamoxifen treatment of *Apln-CreER;Rosa26^{mTmG/+}* mice at P1.5 and analyzed at P3. Region **(B)** represents the luminal surface and contains unremodeled trabecular myocardium that persists at this stage. Yellow arrowheads indicate PECAM⁺ endothelial cells that are not labeled by *Apln-CreER* and that reside on the basement membrane, indicative of endocardial cells. Region **(C)** represents the inner myocardial wall, containing PECAM⁺ endothelial cells that are labeled by *Apln-CreER* and that reside within myocardium rather than on COL3A1 basement membrane, identifying them as tubelike coronary VECs (white arrowheads). White scale bar, 100 μ m, yellow scale bar, 10 μ m.



basement membrane at P0 (fig. S11, B and C). However, at P7, endocardial cells and their derivatives (labeled by Tie2-Cre or VE Cad-CreER) were no longer aligned with COL3A1 strands but rather took up intramyocardial positions (P7 in fig. S11, B and C). To better visualize the process by which P0 endocardium transforms into intramyocardial VECs at P7, we studied an intermediary stage (P3) when hearts contain both unremodeled trabeculae (Fig. 4B) and actively compacting regions (Fig. 4C). We labeled developmental intermediates by treating *Apln-CreER; Rosa26^{mtmG/+}* mice with tamoxifen at P1.5 (Fig. 4A). Trapped endothelial cells remote from the basement membrane adopted the morphology of individual capillary-like cells and were marked by the VEC genetic lineage tracer (green fluorescent protein, see GFP in Fig. 4C), whereas endothelial cells facing the ventricular lumen and residing on the basement membrane retained sheetlike morphology and did not express VEC lineage tracer, consistent with endocardial identity (Fig. 4B). These observations suggest that myocardial compaction traps sheets of endocardial cells, which convert to the VEC lineage and translocate to an intramyocardial location.

We investigated conditions that might promote endocardium to VEC transition in the neonatal heart. Hypoxypromote, a hypoxia-sensitive chemical probe, indicated that rapid expansion of the compact myocardium by trabecular coalescence in the first several postnatal days of life creates a hypoxic environment within the inner myocardial wall (fig. S12). Expression of hypoxia inducible factor 1 α (*Hif1 α*) and vascular endothelial growth factor A *Vegfa*, genes known to be up-regulated by hypoxia, increased in the inner myocardial wall of the P1 and P3 neonatal hearts (fig. S12, B and C). This corresponds to the region in which we observed endocardial to VEC lineage conversion, suggesting that hypoxia and its resulting up-regulation of the key angiogenic factor *Vegfa* contribute to this process.

Our work reveals a mechanism by which trabecular coalescence and endocardial-to-VEC lineage conversion drive vascular expansion in the postnatal heart. Why does coronary vascular growth in this setting rely on this alternative mechanism, rather than occurring through more typical angiogenic sprouting from preexisting vessels? The transition from fetal to postnatal circulation acutely increases the hemodynamic burden on the left ventricle. To accommodate this increased workload, we reason that mammals developed trabecular myocardium as a reservoir of new cardiomyocytes that is quickly recruited after birth through myocardial compaction to increase neonatal left ventricular mass. In addition to cardiomyocytes, this myocardial reservoir also contains coronary vessel precursors in the form of endocardial cells. Trabeculae coalesce during neonatal myocardial compaction causes regional hypoxia that stimulates the trapped neonatal endocardial cells to form the vascular supply for the newly compacted myocardium. This mechanism likely allows more rapid vascular and myocardial growth than angiogenic sprouting of the first CVP from the periphery.

Understanding this endogenous mechanism for rapidly developing a functional vascular supply has important implications for cardiac diseases and cardiac regenerative medicine (4).

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SUPPLEMENTARY MATERIALS

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Figs. S1 to S12
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PLANT-FUNGAL ECOLOGY

Niche engineering demonstrates a latent capacity for fungal-algal mutualism

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Mutualistic symbioses shape the evolution of species and ecosystems and catalyze the emergence of biological complexity, yet how such symbioses first form is unclear.

We show that an obligate mutualism between the yeast *Saccharomyces cerevisiae* and the alga *Chlamydomonas reinhardtii*—two model eukaryotes with very different life histories—can arise spontaneously in an environment requiring reciprocal carbon and nitrogen exchange. This capacity for mutualism is phylogenetically broad, extending to other *Chlamydomonas* and fungal species. Furthermore, we witnessed the spontaneous association of *Chlamydomonas* algal cells physically interacting with filamentous fungi. These observations demonstrate that under specific conditions, environmental change induces free-living species to become obligate mutualists and establishes a set of experimentally tractable, phylogenetically related, synthetic systems for studying the evolution of symbiosis.

Mutualistic symbioses—beneficial associations between different species involving persistent physical contact and physiological coupling—are central to many evolutionary and ecological innovations (1–3). These include the origin of eukaryotic cells, the colonization of land by plants, coral reefs, and the gut microbiota of insects and animals (4, 5). Despite their ubiquity and importance, we understand little about how mutualistic symbioses form between previously free-living organisms (5, 6). Like speciation, the birth of novel symbioses has rarely been witnessed, making it

difficult to determine if coevolution occurs before symbiosis begins or if chance ecological encounters initiate new symbioses (5, 7). Such “ecological fitting” (8, 9) occurs when both a particular environment and previously evolved traits allow a set of species to complement each other, giving rise to novel interactions without the need for prior coevolutionary adaptation.

We tested two genetically tractable organisms, the budding yeast *Saccharomyces cerevisiae* and the green alga *Chlamydomonas reinhardtii*, to determine if a reciprocal exchange of carbon and nitrogen would lead to obligate mutualism between algae and fungi such as those that occur naturally (10–13). In our scheme (Fig. 1A), *S. cerevisiae* metabolizes glucose to carbon dioxide (CO₂), a carbon source that *C. reinhardtii* fixes via photosynthesis, and *C. reinhardtii* reduces nitrite (NO₂[−]) into ammonia (NH₃) (14), which yeast can use as a nitrogen source. Coculturing

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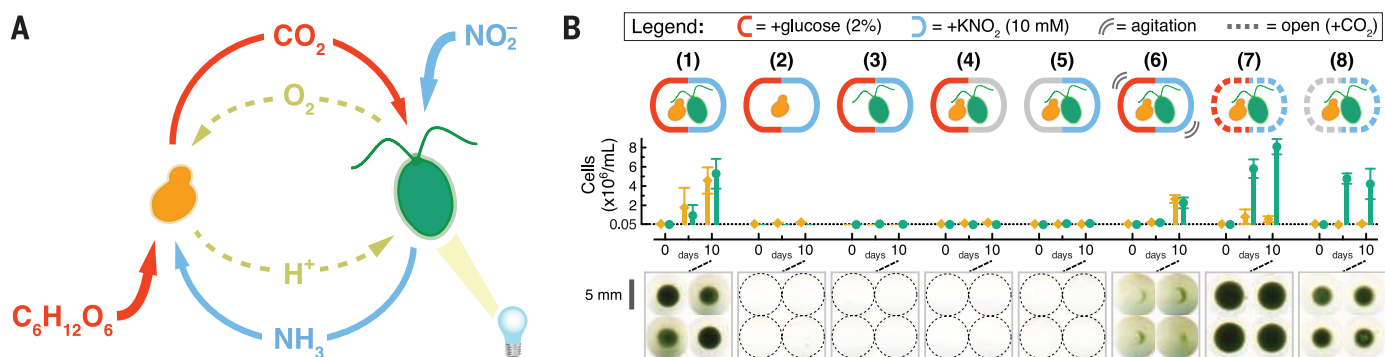


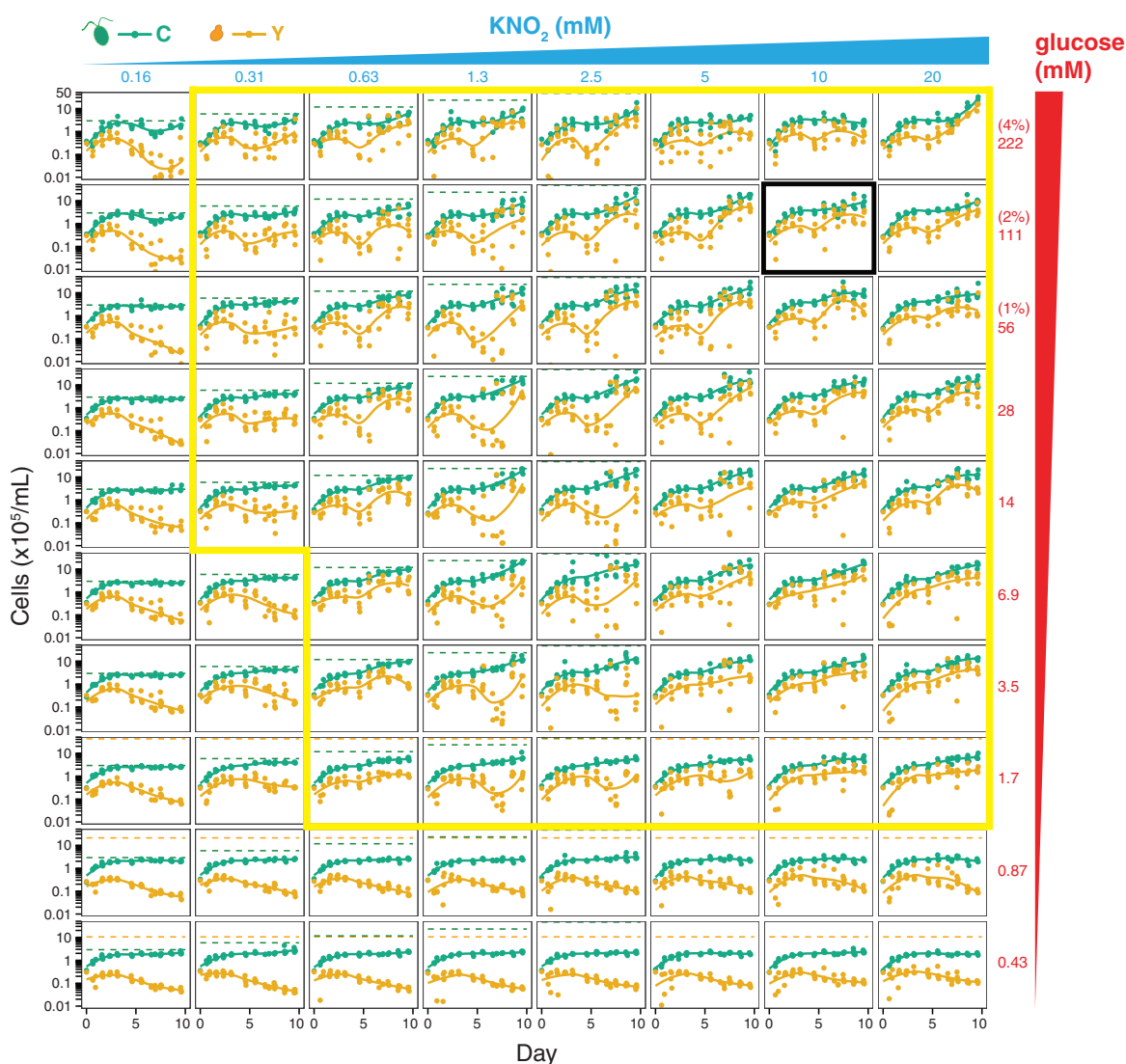
Fig. 1. A synthetic mutualism between *S. cerevisiae* and *C. reinhardtii*.

(A) A metabolic circuit for mutualism based on carbon and nitrogen exchange. *S. cerevisiae* (orange, left) metabolizes glucose ($C_6H_{12}O_6$) and releases carbon dioxide (CO_2), which is assimilated photosynthetically by *C. reinhardtii* (green, right) to release oxygen (O_2); *C. reinhardtii* metabolizes nitrite (NO_2^-) and releases ammonia (NH_3) as a nitrogen source for *S. cerevisiae*. An intrinsic, near-neutral pH balance between 6.8 and 7.4 is maintained by a metabolic exchange of protons between yeast and alga. (B) Proliferation of *S. cerevisiae* and

C. reinhardtii under different coculture conditions demonstrates that obligate mutualism can arise without any genetic engineering of metabolic pathways. Top: cartoons of the different conditions tested; middle: cell density of yeast and alga over the course of the experiment (mean $\pm$ 95% confidence interval; $N = 4$); bottom: images of the cell populations from four representative examples of each culture condition (after 10 days). The dark green hue of the pellets is due to *C. reinhardtii* cells; *S. cerevisiae* cells are off-white and are interspersed throughout the pellet. See (15) for further details.

Fig. 2. Landscape of mutualistic productivity.

Cell densities over time for each species grown in coculture (*C. reinhardtii* in green; *S. cerevisiae* in orange) grown from an initial inoculum of $\sim 0.3 \times 10^5$ cells/mL for each species; irradiance = $110 \mu\text{mol m}^{-2} \text{s}^{-1}$. Each of four replicate point pairs (green and orange) is plotted. Local polynomial regression fits (by robust linear regression in R with $y \sim x$) for both cell types are plotted as a visual guide of cell proliferation. Coculture conditions are denoted on the top and right and show increasing (left to right) KNO_3 concentration and increasing (bottom to top) glucose concentration. The standard conditions of 10 mM KNO_3 and 111 mM (2%) glucose used in Fig. 1 are outlined by a black box. Dashed lines indicate the maximum predicted cell densities expected for *C. reinhardtii* (green) and *S. cerevisiae* (orange) for each coculture (15). Net positive proliferation of both yeast and algae is supported within the region bounded by the yellow outline. The limited proliferation of *C. reinhardtii* under conditions outside this region (in days 1 and 2) indicates residual atmospheric CO_2 in the wells of the sealed microtiter plate.



experiments (15) indicate that by preventing access to atmospheric CO₂, *S. cerevisiae* and *C. reinhardtii* become obligate mutualists (Fig. 1B). This mutualism depends on the metabolic capabilities of the two organisms: *S. cerevisiae* cannot use nitrite as a nitrogen source and *C. reinhardtii* cannot use glucose as a carbon source. Cell proliferation did not require genetic engineering or fine-tuning of nutrient concentrations or starting ratios of the two species (Fig. 1B and figs. S1 and S2) and failed when either species (Fig. 1B, conditions 2 and 3), glucose, or nitrite was omitted from the experiment (Fig. 1B, conditions 4 and 5). Agitation attenuates this mutualism (Fig. 1B, condition 6), suggesting the importance of cell-cell proximity and spatial structure in establishing successful cooperation (16). Thus, a simple environmental change can induce free-living organisms to be mutualistic without requiring adaptive coevolution.

In our scheme, mutualism can be obligate or facultative depending on the environment. Access to atmospheric CO₂ makes *C. reinhardtii* a facultative mutualist by removing its dependence on *S. cerevisiae* for carbon (Fig. 1B, condition 7), but the yeast remains dependent on the alga for

nitrogen. In this environment, algal proliferation is improved by the presence of glucose-metabolizing, CO₂-generating budding yeast whereas yeast proliferation is reduced, although not extinguished (Fig. 1B, compare conditions 7 and 8). Conversely, adding ammonia (as ammonium chloride) to airtight cocultures allows budding yeast to proliferate independently of the alga whereas the alga remains dependent on the yeast for carbon. Under these conditions, *S. cerevisiae* (~4 hours doubling time in our conditions) outcompetes *C. reinhardtii* (≥12 hours doubling time) and drives the alga to near extinction (fig. S1, condition 15). These results suggest that stable metabolic mutualisms require that the faster-growing species be obligately dependent on nutrients produced by its slower-growing partner.

The engineered obligate mutualism between *S. cerevisiae* and *C. reinhardtii* is not limited to our initial choice of input nutrient concentrations. Successful mutualisms were established over nearly two orders of magnitude in glucose and nitrite concentrations (Fig. 2). However, this resulted in complex population dynamics. We observed undulations and variations in stability across time similar to density-dependent popu-

lation cycles predicted for mutualistic systems (17). Other carbon (e.g., galactose) or nitrogen (e.g., nitrate) sources, although less effective, also sustain mutualism between *S. cerevisiae* and *C. reinhardtii* (fig. S3).

We also demonstrate that many different ascomycetous yeast and four *Chlamydomonas* species, spanning over 300 million years of evolutionary divergence in each clade, can form mutualisms (Fig. 3). Nearly all yeast species we examined form synthetic obligate mutualisms with *C. reinhardtii*, although with different degrees of productivity (Fig. 3A). Mutualistic productivity, as assessed by total cell counts, did not correlate with a yeast's preference for a fermentative or respiratory lifestyle (Fig. 3A), whether a yeast strain was isolated from soil (a potential habitat shared with *C. reinhardtii*), intrinsic growth rate, or nitrite-mediated inhibition of growth (fig. S4 and table S1). Thus, we observe that mutualisms can be phylogenetically broad, but that the degree of success depends on species-specific traits.

Two yeast species and the alga *Chlorella vulgaris* did not form obligate mutualisms (Fig. 3). *C. vulgaris*, which can use glucose as a carbon source, outcompeted *S. cerevisiae*, whereas

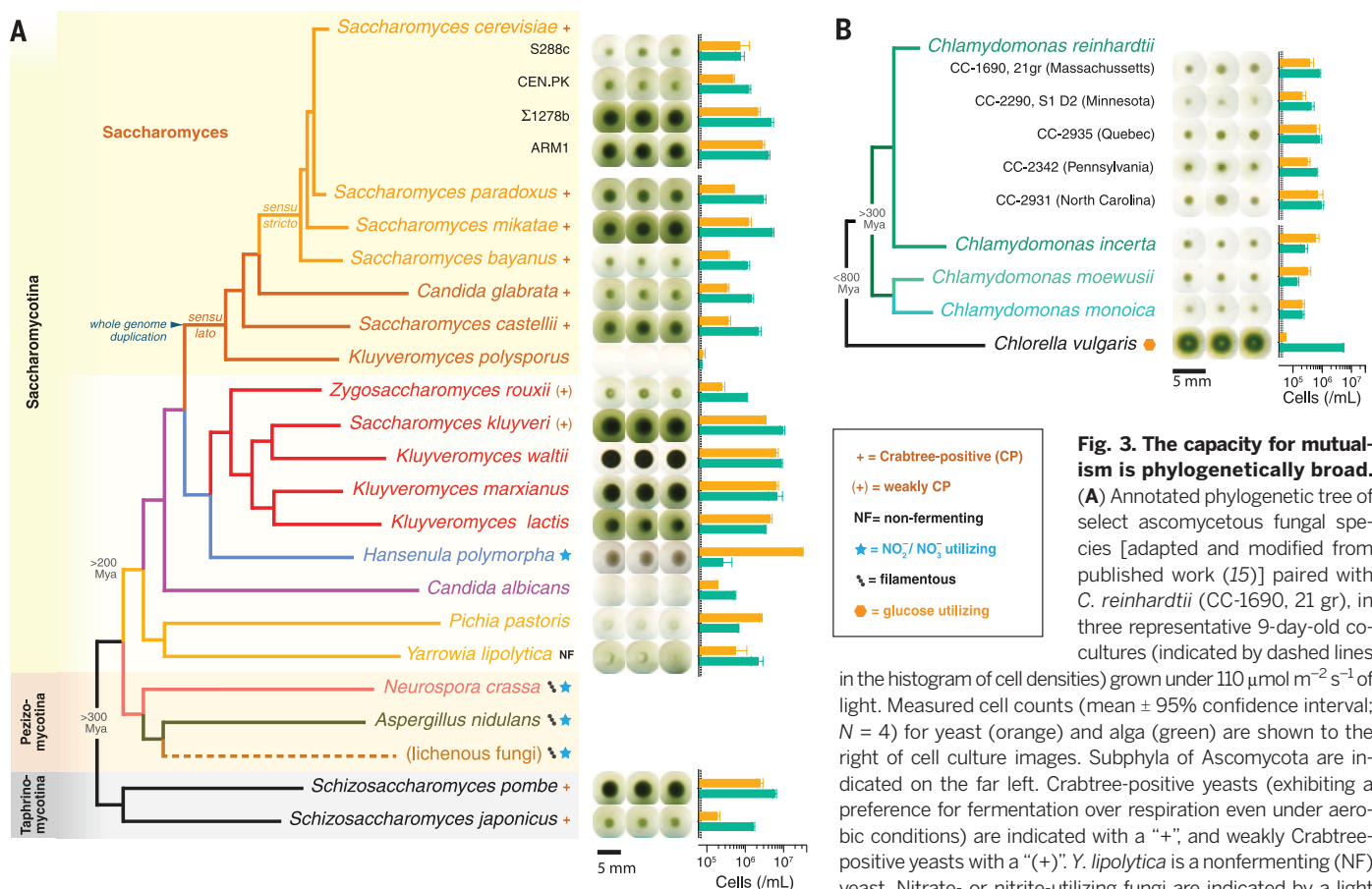


Fig. 3. The capacity for mutualism is phylogenetically broad.

(A) Annotated phylogenetic tree of select ascomycetous fungal species [adapted and modified from published work (15)] paired with *C. reinhardtii* (CC-1690, 21 gr), in three representative 9-day-old cocultures (indicated by dashed lines in the histogram of cell densities) grown under 110 μmol m⁻² s⁻¹ of light. Measured cell counts (mean ± 95% confidence interval; N = 4) for yeast (orange) and alga (green) are shown to the right of cell culture images. Subphyla of Ascomycota are indicated on the far left. Crabtree-positive yeasts (exhibiting a preference for fermentation over respiration even under aerobic conditions) are indicated with a "+", and weakly Crabtree-positive yeasts with a "(+)". *Y. lipolytica* is a nonfermenting (NF) yeast. Nitrate- or nitrite-utilizing fungi are indicated by a light blue star and filamentous fungi by three connected dots (see fig. S5). (B) Annotated phylogenetic tree [adapted and modified from published work (15)] of select algal species and *Chlamydomonas* cultivars (green bars at right) paired with *S. cerevisiae* (S288C) (orange bars, right) in representative 7-day-old cocultures grown under 110 μmol m⁻² s⁻¹ of light (mean ± 95% confidence interval; N = 12). *Chlorella vulgaris* is an asexual alga, distantly related to *C. reinhardtii*, and able to use glucose as a carbon source (orange hexagon). Descriptions of strains are provided in table S1.

Hansenula polymorpha, a yeast that can use nitrite as a sole nitrogen source, outperformed *C. reinhardtii*. The yeast *Kluyveromyces polysporus* failed to form an obligate mutualism with *C. reinhardtii*. This yeast can grow in an ammonium-supplemented coculture medium, suggesting that it fails to cooperate with *C. reinhardtii* likely because it either cannot grow at the low ammonia levels produced by *C. reinhardtii* or is more sen-

sitive to nitrite inhibition at such low ammonia levels (fig. S4). *Neurospora crassa* and *Aspergillus nidulans* are genetically tractable filamentous fungi that can use nitrite as a nitrogen source (18). The ability of these fungi to reduce nitrite keeps wild-type strains from forming obligate mutualisms with *C. reinhardtii*. However, mutants that cannot reduce nitrite did form obligate mutualisms (fig. S5), suggesting that a loss of

gene function in one species could be complemented through mutualism (7, 19).

We observed that the filamentous fungi formed macroscopic structures (fig. S5) such that the fungal hyphae were decorated with *C. reinhardtii* cells (Fig. 4, A and B, and movies S1 to S6). However, physical associations between fungus and alga form even in the absence of any metabolic dependency (figs. S6 and S7 and movies S7 to S16). Electron microscopy of interactions between *C. reinhardtii* and *A. nidulans*, which shares a most recent common ancestor with lichenous fungi within the class Eurotiomycetes (10), revealed a tight fungal-algal contact interface (Fig. 4, C and D) reminiscent of wall-to-wall interfaces between fungal and algal cells in extant lichens (20). The walls of *C. reinhardtii* cells in contact with *A. nidulans* hyphae are less heavily stained and appear thinner than *C. reinhardtii* cells cultured separately (Fig. 4E), possibly because of locally secreted *A. nidulans* cell wall-remodeling enzymes. We saw no evidence of any morphologically complex tissue structures, such as those seen in many lichens, nor of fungal hyphae penetrating algal cells (11, 20). Thus, these synthetic mutualisms may result in physical complexes but they do not appear to form elaborate morphological structures at the cellular or organismal level.

The ease with which fungal-algal mutualisms were created suggests that ecological interactions may be relatively easy to establish (21). Furthermore, they do not require a prior facultative, commensal, or parasitic stage, or coevolutionary adaptation (5–7, 22, 23). Our understanding of how “ecologically framed” pairs of species can be created in response to environments that force them to depend on each other will be useful in the emerging field of synthetic ecology (24, 25), as well as for understanding the assembly of microbial communities in cases of disturbed or invaded habitats.

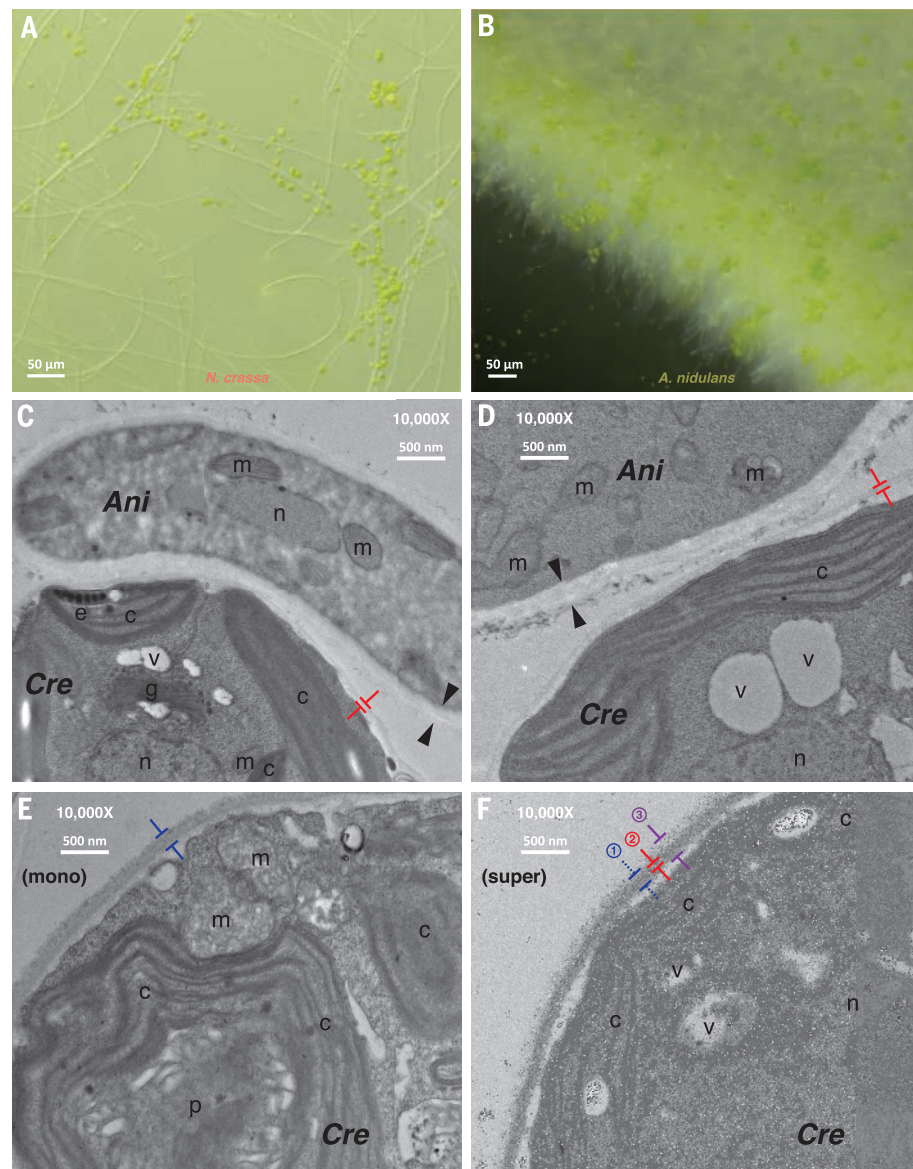


Fig. 4. *C. reinhardtii* physically associates with *N. crassa* and *A. nidulans*. Representative light micrographs of the periphery of fungal-algal associations formed in obligate mutualistic coculture. *C. reinhardtii* cells (green) stick to hyphae (white filaments) of (A) *N. crassa* (FGSC 11007 $\Delta nit-4$) or (B) *A. nidulans* (TS003 *crnA*–*crnB*). (C to F) Representative transmission electron micrographs reveal a simple wall-to-wall interface between *C. reinhardtii* (Cre) cells and *A. nidulans* (Ani) hyphae. Opposed arrows indicate the thickness of fungal cell walls, and opposed colored T-bars indicate those of algal cells [(C): 51 ± 10 nm; (D): 60 ± 7 nm; mean $\pm$ SD]. (E) *C. reinhardtii* grown in monoculture (160 ± 20 nm; blue T-bars) or (F) unattached *C. reinhardtii* isolated from the supernatant of the same coculture {T-demarcations: reference monoculture cell wall thickness [blue dashed; see (E)]; (2) core (heavy) cell wall staining (red): 50 ± 4 nm; (3) diffuse cell wall staining (purple): 260 ± 30 nm} (15). Labeled intracellular components: c, chloroplast; e, eyespot; g, Golgi; m, mitochondria; n, nucleus; p, pyrenoid; and v, vacuole.

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materials. E.F.Y.H. conceived the project, performed the experiments, and analyzed the data. E.F.Y.H. and A.W.M. devised the research and wrote the manuscript. A.W.M. supported and provided input throughout all stages of this work.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6192/94/suppl/DC1
Materials and Methods

Figs. S1 to S7

Tables S1 to S8

Movies S1 to S16

References (26–71)

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CELL DEATH

Opposing unfolded-protein-response signals converge on death receptor 5 to control apoptosis

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Protein folding by the endoplasmic reticulum (ER) is physiologically critical; its disruption causes ER stress and augments disease. ER stress activates the unfolded protein response (UPR) to restore homeostasis. If stress persists, the UPR induces apoptotic cell death, but the mechanisms remain elusive. Here, we report that unmitigated ER stress promoted apoptosis through cell-autonomous, UPR-controlled activation of death receptor 5 (DR5). ER stressors induced *DR5* transcription via the UPR mediator CHOP; however, the UPR sensor IRE1 α transiently catalyzed *DR5* mRNA decay, which allowed time for adaptation. Persistent ER stress built up intracellular DR5 protein, driving ligand-independent DR5 activation and apoptosis engagement via caspase-8. Thus, DR5 integrates opposing UPR signals to couple ER stress and apoptotic cell fate.

The endoplasmic reticulum (ER) mediates folding and maturation of transmembrane and secreted proteins (1, 2). Elevated physiological demand for protein folding can cause misfolded proteins to accumulate in the ER lumen—a condition called ER stress. The unfolded protein response (UPR) senses such stress and mediates cellular adaptation by expanding the ER's protein-folding capacity while decreasing its synthetic load. Protein kinase R (PKR)-like kinase (PERK) and inositol-requiring enzyme 1 α (IRE1 α) are two key metazoan UPR sensors (1, 2); residing in the ER membrane, each has a luminal domain that detects misfolded polypeptides. PERK harbors a cytoplasmic kinase moiety that phosphorylates eukaryotic translation-

initiation factor 2 α (eIF2 α). This suppresses general translation but promotes synthesis of preferred factors—including ATF4, which activates the UPR transcription factor CCAAT/enhancer-binding protein homologous protein (CHOP), among other genes. IRE1 α has both kinase and endoribonuclease (RNase) cytoplasmic moieties (3). The kinase controls RNase activity, which mediates regulated IRE1 α -dependent decay (RIDD) of ER-associated mRNAs (4) and generates the UPR transcription factor X-box binding protein 1 spliced (XBPs). Certain pathological conditions can cause irreversible ER stress (5), often leading to apoptotic cell death (1, 2, 6). Two interconnected signaling cascades control apoptosis: the intrinsic, mitochondrial pathway, and the extrinsic, death-receptor pathway (7). Each engages distinct proteases, called initiator caspases, to activate a common set of executioner caspases (8). Unmitigated ER stress regulates the intrinsic pathway via several Bcl-2 family proteins (1, 2, 6, 9, 10). Furthermore, IRE1 α cleaves specific micro-RNAs to derepress caspase-2 expression (11); however, caspase-2 may be dispensable for ER stress-induced apoptosis (12), which leaves the underlying initiation mechanisms obscure.

Experiments with biological and pharmacological ER stressors revealed consistent activation of caspase-8, the pivotal initiator in the extrinsic pathway (8) (Fig. 1). The bacterial AB5 subtilase cytotoxin SubAB induces pathophysiological ER stress by cleaving the chaperone BiP (13). SubAB caused dose-dependent BiP depletion and ER stress, evident by CHOP and XBPs up-regulation, in KMS11 multiple myeloma cells (Fig. 1A). In keeping with data that PERK activity persists, whereas IRE1 α activation is transient (14), CHOP remained elevated, whereas XBPs declined by 24 hours. SubAB also induced activation of caspase-8 and caspase-3 by 24 hours, evident by cleaved caspase and poly(ADP ribose) polymerase (PARP) products. SubAB substantially increased caspase-8 and caspase-3/7 enzymatic activity, and DNA fragmentation—an apoptotic hallmark (fig. S1, A to C). Brefeldin-A (BFA)—an inhibitor of ER-to-Golgi trafficking—similarly induced ER stress, caspase activation, and apoptosis in SK-MES-1 lung carcinoma cells (Fig. 1B and fig. S1, D to F). The sarcoplasmic ER calcium-adenosine triphosphatase inhibitor thapsigargin (Tg) induced persistent CHOP and transient XBPs expression in wild-type and in *Bax*^{−/−} HCT116 colon carcinoma cells; whereas apoptosis required *Bax*, caspase-8 activation did not (Fig. 1, C and D, and fig. S1, G to I). Moreover, small interfering RNA (siRNA) depletion of caspase-8, but not caspase-2, blocked activation of caspase-3/7 and apoptosis by diverse ER stressors (Fig. 1, E and F, and fig. S1, J to O). Caspase-8 activates the Bcl-2 family protein Bid to engage the intrinsic pathway via Bax (15, 16). Full-length Bid declined in association with Tg-induced caspase-8 activation (fig. S1I), which indicated Bid processing. Bid siRNA knockdown commensurately attenuated Tg-induced apoptosis, whereas caspase-8 siRNA inhibited both Bid processing and apoptosis (fig. S1, P to S). Tg also up-regulated Bim (fig. S1I) as reported (10); however, caspase-8 and Bid processing occurred much earlier, which suggests that Bim might support later apoptotic signals. Thus, caspase-8 plays a pivotal role, whereas caspase-2 appears dispensable, during apoptosis induction by unmitigated ER stress.

Upon binding of cognate extracellular ligands, the death receptors Fas, DR4, or DR5 nucleate a death-inducing signaling complex (DISC) at the plasma membrane, which activates caspase-8 via

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the adaptor Fas-associated death domain (FADD) (17). Consistent with evidence that ER stress up-regulates *DR5* transcription (18), quantitative reverse transcription polymerase chain reaction (QPCR) showed a two- to fourfold *DR5* mRNA induction by Tg, BfA, SubAB, or the glycosylation inhibitor tunicamycin (Tm), with less impact on *DR4*, *Fas*, or *TNFR1* (Fig. 2, A and B, and fig. S2, A to C). ER stressors most often elevated both the long (DR5L) and short (DR5S) splice variants of DR5 (Fig. 2C and fig. S2, D to H) (19). Tg up-

regulated DR5 within 6 hours, in concert with CHOP and XBP1s induction, yet preceding caspase-8 processing (Fig. 2C and fig. S2D). Furthermore, Tg induced a DR5-nucleated complex with FADD and caspase-8, harboring elevated caspase-8 activity, independent of *Bax* (Fig. 2D and fig. S2, I to K). Immunoprecipitation (IP) of DR5 or caspase-8 showed comparable caspase-8 activity (fig. S2L). Consistently, other ER stressors increased DR5-associated caspase-8 activity in multiple cell lines (fig. S2, M to O). Livers from

Tm-treated mice also showed elevated DR5 and cleaved caspase-8 in conjunction with apoptosis (fig. S2, P and Q). DR5 siRNA knockdown in different cell lines strongly inhibited caspase activation and apoptosis in response to various ER stressors (Fig. 2, E to H, and fig. S2, R to X). Thus, DR5 is critical for caspase-8-mediated apoptotic engagement by unmitigated ER stress.

Remarkably, siRNA depletion of the sole DR5 ligand, Apo2L/TRAIL (apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand), had no impact on Tg-induced apoptosis in HCT116 or SK-MES-1 cells, unlike caspase-8 knockdown (Fig. 3A and fig. S3, A and B). Moreover, neutralization of extracellular Apo2L/TRAIL by using soluble DR4- and DR5-Fc fusion proteins, which blocked exogenously added ligand, did not inhibit apoptosis activation by Tg or BfA (Fig. 3B and fig. S3, C and D). Thus, ER stress induces ligand-independent DR5 activation. DR5 was barely detectable by immunofluorescence in resting SK-MES-1 cells but showed higher abundance with the addition of Tg or BfA (Fig. 3C). In Tg-treated cells, DR5 colocalized with the Golgi marker RACS1 but not the ER marker KDEL; however, in BfA-treated cells—which had minimal Golgi—DR5 did colocalize with KDEL (Fig. 3D and fig. S3, E and F). Despite massively elevating total DR5, BfA did not substantially up-regulate cell surface DR5, nor did it increase sensitivity to exogenous Apo2L/TRAIL (fig. S3, G to J). However, Tg, which up-regulated both total and cell surface DR5, did enhance sensitivity to added ligand (fig. S3, K to N). DR5 partially colocalized with cleaved caspase-8 within Tg-treated cells (fig. S3O), which supports intracellular activation. Size-exclusion chromatography of detergent extracts from HCT116 cells revealed Tg-driven up-regulation of DR5L and DR5S in low molecular weight (MW) fractions—representing DR5 oligomers; elevated DR5L appeared also in high

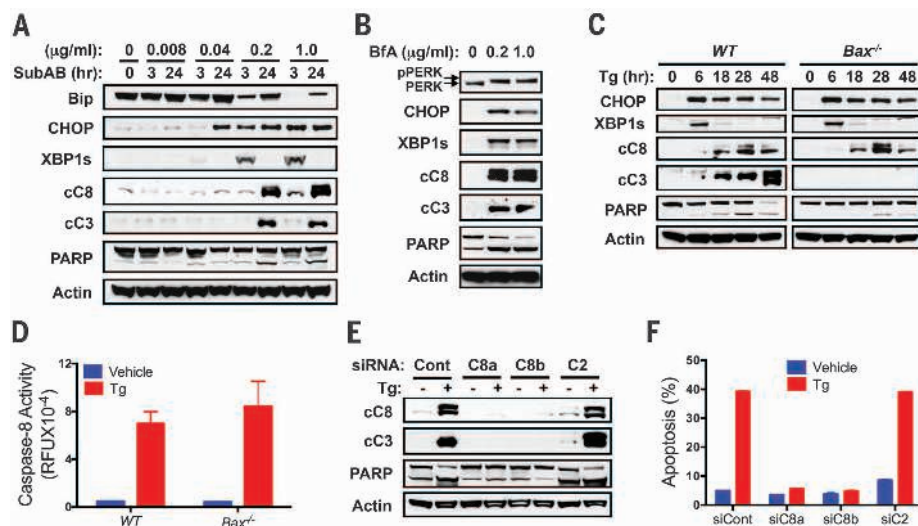


Fig. 1. Unmitigated ER stress triggers apoptosis via caspase-8. (A) KMS11 cells were treated with SubAB and analyzed by immunoblot. cC8: cleaved caspase-8; cC3: cleaved caspase-3. (B) SK-MES-1 cells were treated with BfA (24 hours) and analyzed by immunoblot. (C and D) Wild-type (WT) or *Bax*^{-/-} HCT116 cells were treated with Tg (100 nM) and analyzed by immunoblot (C), or caspase-8 activity assay (24 hours) (D). (E and F) HCT116 cells were transfected (48 hours) with control siRNA (Cont), a single (C8a), or an independent pool (C8b) of caspase-8 siRNAs, or caspase-2 siRNA. Cells were treated with Tg (100 nM, 24 hours) and analyzed by immunoblot (E) or FACS to measure apoptosis by subG₁ DNA content (F). Graphs depict means ± SD of triplicates (D) or duplicates (F).

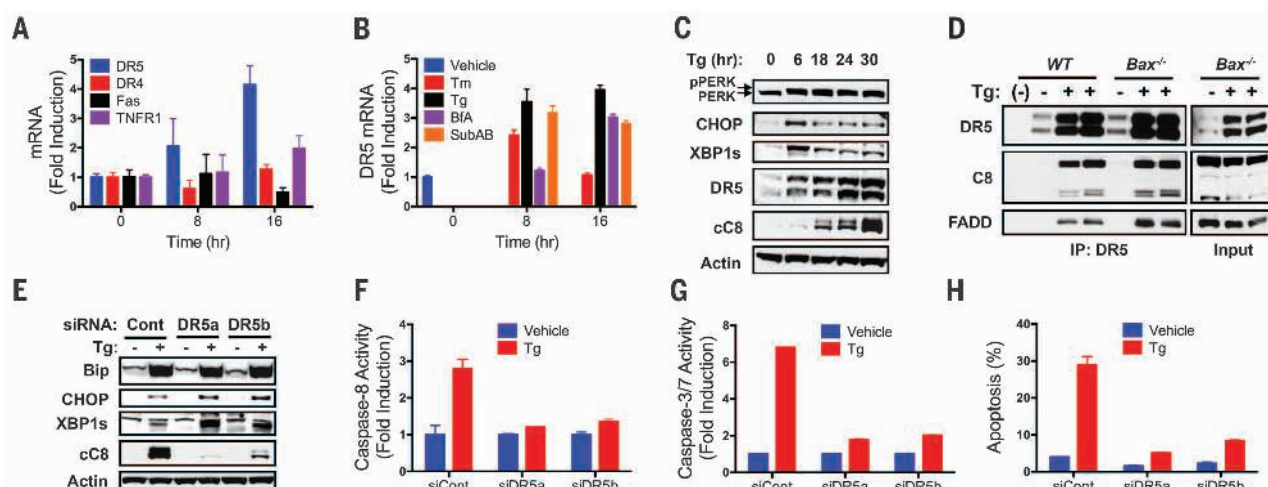


Fig. 2. Unmitigated ER stress activates caspase-8 via DR5. (A) HCT116 cells were treated with Tg (100 nM), and mRNA levels were measured by QPCR [normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH)]. (B) HCT116 cells were treated with Tm (1 μg/ml), Tg (100 nM), BfA (1 μg/ml), or SubAB (1 μg/ml) and analyzed by QPCR (normalized to GAPDH). (C) HCT116 cells were treated with Tg (100 nM) and analyzed by immunoblot. (D) WT or

Bax^{-/-} HCT116 cells were treated as in (C) (24 hours), subjected to DR5 IP, and analyzed by immunoblot. (E to H) HCT116 cells were transfected (48 hours) with control siRNA (Cont), a single (DR5a), or an independent pool (DR5b) of DR5 siRNAs; then treated with Tg (100 nM, 24 hours) and analyzed by immunoblot (E), by enzymatic activity assay for caspase-8 (F) or caspase-3/7 (G), or by FACS for apoptosis (H). Graphs depict means ± SD of triplicates.

MW fractions—which indicates the presence of DR5L multimers (Fig. 3E). Caspase-8 activity occurred in two peaks: one coinciding with DR5L in high MW fractions, the other separate from DR5 in low MW compartments (Fig. 3F). Chemical cross-linking verified Tg-induced formation of DR5 oligomers and multimers (fig. S3P). Furthermore, selective DR5L siRNA knockdown attenuated Tg-driven activation of caspase-8 and apoptosis (fig. S3, Q to T). Thus, Tg up-regulates both DR5 variants but DR5L preferentially

multimerizes, recruiting and activating caspase-8 and releasing processed enzyme into lower MW fractions.

Consistent with earlier evidence (18, 20), siRNA depletion of CHOP substantially blocked *DR5* mRNA up-regulation by Tg or BfA, whereas knockdown of the CHOP transcriptional targets ER oxidase 1 α (ERO1 α) or growth arrest and DNA damage-inducible 34 (GADD34) did not (Fig. 4A and fig. S4, A to E); these findings support direct CHOP control of *DR5* mRNA. Although

GADD34 dephosphorylates eIF2 α to reinitiate translation, ERO1 α is important for protein disulfide isomerization and folding in the ER lumen (6). ERO1 α knockdown did inhibit DR5L protein up-regulation and the associated apoptotic events (fig. S4, F to H), which suggests that ERO1 α may facilitate folding of DR5L (DR5L harbors more cysteine residues than DR5S). In contrast to CHOP depletion, siRNA knockdown of IRE1 α attenuated *DR5* mRNA decay in Tg-treated cells (Fig. 4B and fig. S4, I to L), which suggests that IRE1 α counter-

Fig. 3. Unmitigated ER stress engages caspase-8 by inducing ligand-independent intracellular DR5 activation.

(A) HCT116 cells were transfected (48 hours) with control siRNA or siRNA targeting Apo2L/TRAIL or caspase-8. Cells were treated with Tg (100 nM, 24 hours) and analyzed for apoptosis. (B) HCT116 cells were treated (24 hours) with Apo2L/TRAIL (1 μ g/ml) or Tg (100 nM) in the presence of vehicle or DR4-Fc plus DR5-Fc (10 μ g/ml each) and analyzed for apoptosis. (C) SK-MES-1 cells were treated (16 hours) with indicated ER stressors and analyzed by immunofluorescence with DR5-specific antibody. (D) SK-MES-1 cells were treated with Tg (20 nM, 24 hours) and analyzed by immunofluorescence for DR5 or RACS1. (E and F) HCT116 cells were treated with Tg (100 nM, 24 hours), extracted with 1% Triton X-100, and subjected to size-exclusion chromatography; fractions were analyzed by DR5 IP and immunoblot (E) or caspase-8 activity assay (F). Control: direct DR5 IP from Tg-treated cells. Graphs depict means $\pm$ SD of duplicates (B) or triplicates (F). Scale bars: 20 μ m (C and D).

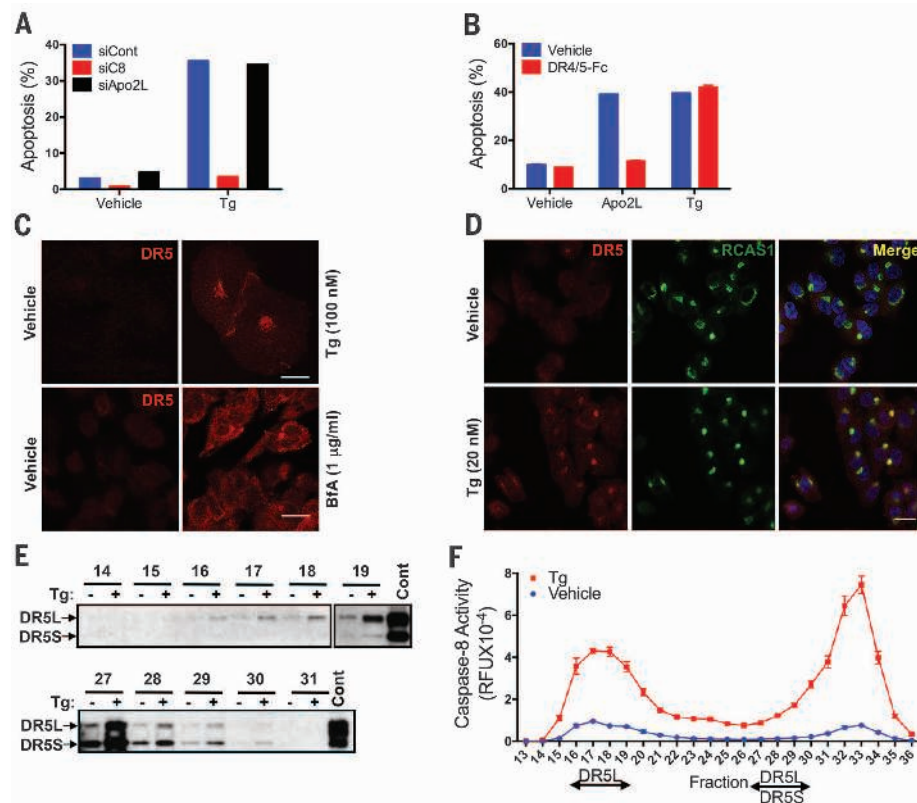
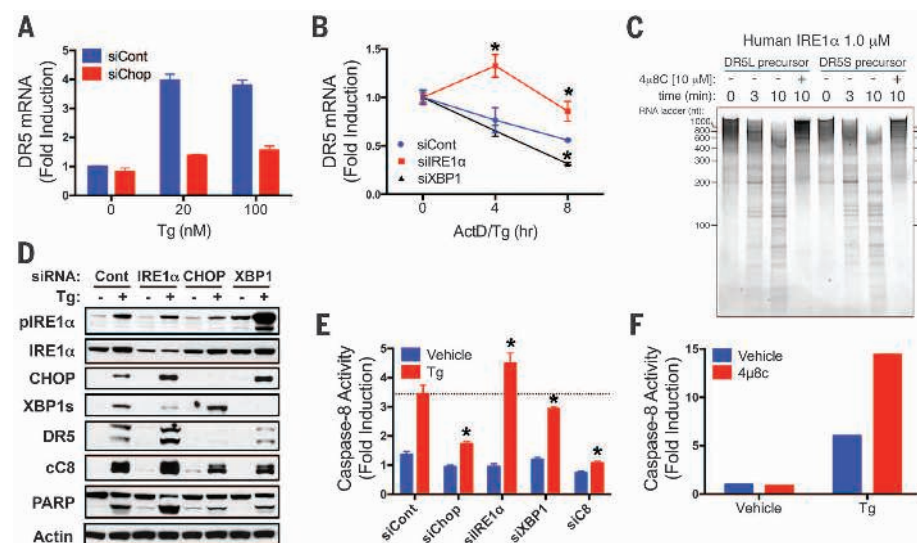


Fig. 4. DR5 integrates opposing UPR signals to control apoptosis.

(A) HCT116 cells were transfected (48 hours) with control or CHOP siRNA, treated with Tg (8 hours), and analyzed by QPCR (normalized to GAPDH). (B) HCT116 cells were transfected (48 hours) with control, IRE1 α , or XBP1 siRNA; and treated with actinomycin D (2 μ g/ml) plus Tg (20 nM), then *DR5* mRNA was measured as in (A). (C) Purified recombinant human IRE1 α comprising the kinase and RNase domains (KR43) was incubated with in vitro transcribed *DR5L* or *DR5S* mRNA in the presence of vehicle or 4 μ 8C (10 μ M). Reactions were resolved on a 6% Tris-borate-EDTA (TBE)-urea polyacrylamide electrophoresis gel and stained with SYBR Gold. (D and E) HCT116 cells were transfected (48 hours) with control, IRE1 α , CHOP, or XBP1 siRNA; treated with Tg (100 nM, 24 hours); and analyzed by immunoblot (D) or caspase-8 activity assay (E). (F) HCT116 cells were treated with Tg (100 nM, 24 hours) in the presence of vehicle or 4 μ 8C (30 μ M) and analyzed for caspase-8 activity. Graphs depict means $\pm$ SD of triplicates.



acts apoptosis by mediating *DR5* RIDD. Indeed, a recombinant protein comprising IRE1 α 's catalytic domains cleaved in vitro transcribed *DR5* mRNAs at discrete sites, and this was blocked by the IRE1 α RNase inhibitor 4 μ 8C (2I) (Fig. 4C and fig. S4M). Furthermore, whereas CHOP siRNA attenuated Tg-induced *DR5* up-regulation, caspase-8 activation, and apoptosis, IRE1 α depletion augmented these events (Fig. 4, D and E, and fig. S4N). Conversely, XBP1s knockdown—which led to compensatory IRE1 α hyperphosphorylation (Fig. 4D) as reported (22)—accelerated *DR5* mRNA decay and diminished *DR5* up-regulation, caspase-8 activation, and apoptosis (Fig. 4, B, D, and E, and fig. S4, J to L and N). Finally, 4 μ 8C enhanced caspase activation by Tg (Fig. 4F and fig. S4O), which confirms an antiapoptotic role for IRE1 α RNase. Thus, CHOP and RIDD exert opposing effects on *DR5* to control caspase-8 activation and apoptosis.

Our data delineate a cell-autonomous mechanism wherein *DR5* integrates dynamic UPR signals to control apoptosis in relation to ER stress (fig. S4P). Upon reversible ER disruption, PERK-CHOP activity induces, whereas RIDD suppresses, *DR5* transcripts. If ER stress resolves, UPR activity subsides, and *DR5* mRNA returns to baseline. However, if ER stress prevails, PERK-CHOP function persists, whereas IRE1 α activity attenuates (14), which permits *DR5* mRNA to rise. *DR5* ac-

cumulation in the ER and Golgi apparatus drives ligand-independent multimerization of *DR5L*, which—consistent with earlier data (23)—has greater propensity to cluster than *DR5S*. ERO1 α , previously implicated in UPR-driven apoptosis (6), facilitates *DR5L* up-regulation, perhaps by supporting disulfide isomerization. *DR5L* provides a DISC-like intracellular platform for caspase-8 recruitment and apoptosis initiation. It was proposed that ER stress augments apoptosis by increasing autocrine death-ligand signaling (18, 24, 25); however, ER disruption would attenuate ligand secretion. Our data reveal that *DR5* acts as an intracellular “gauge” for persistence of ER stress. Opposing controls on *DR5* mRNA synthesis and decay by PERK-CHOP versus IRE1 α define a time window for adaptation, before committing the cell to an apoptotic fate.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S4
References (26–30)

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A giant leap

In 1960, my grandfather ascended to 102,800 feet in a gondola lifted by a polymer balloon and, after saying a brief prayer, jumped. He fell for more than 4 minutes before landing safely in the desert of New Mexico, proving that people could survive the hostility of space, and pioneering technology to protect human life in that environment. Years later, a reporter asked Alan Shepard—the first American in space—if he would have jumped. “Hell no,” he said.

Lately, it feels like I’m making my own giant leap. I’ve spent the past decade building up academic credentials, doing all the things a scientist needs to do to land a faculty appointment. I’ve published frequently in good journals, more each year. I’ve landed competitive funding from respected sources and built a research program that involves postgraduates. I’ve taught several classes and mentored graduate students.

Most of this has been rewarding. But in the overly busy mode that most researchers operate in, I found I was feeling less bullish about academia as I knew it and increasingly uncertain it was the right fit.

I had the benefit of several mentors who encouraged me to explore other career options, and in 2013, I took a half-time appointment with a conservation-focused nonprofit as a scientist with an applied focus. I was tasked with supporting their conservation programs with the best available science, working across fields to bring together insights that could inform conservation practice.

I was surprised to find I enjoyed this work much more than basic research. I could see findings being put to work, and the healthy debate about how to adapt research into practice was invigorating and challenging. I gained a new appreciation for the importance of moving research out of the ivory tower, and for the skills needed to engage in that translational role.

Ironically, at about that time, my academic job searches finally started to yield fruit. I was invited for two faculty interviews, both at top-tier research universities. During one, I entered a pressure-cooker group interview, where faculty members lob hard questions at you while you try not to squirm. One of them asked me, “Jack, fast-forward 35 years. We are celebrating your career and accomplishments at your retirement party. What are we celebrating?”

I gave a safe answer: “I made an impact on my field of



“My grandfather fell for more than 4 minutes before landing safely.”

scholarship. I trained some graduate students who have gone on to successful careers” and so on. First she nodded—but then she banged the table emphatically. “That’s not good enough,” she said.

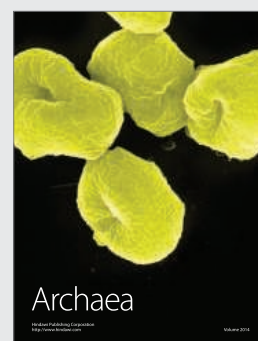
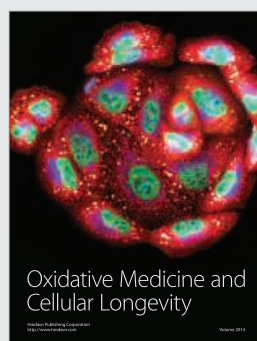
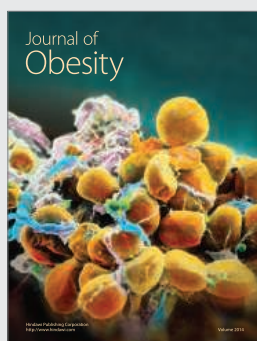
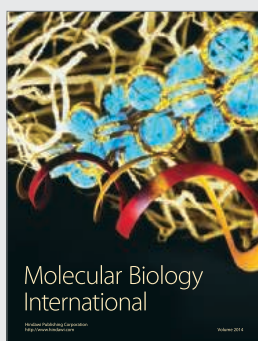
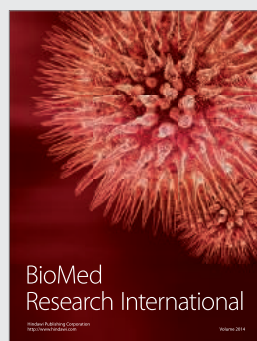
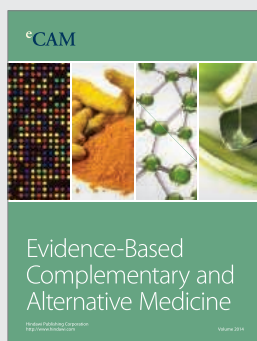
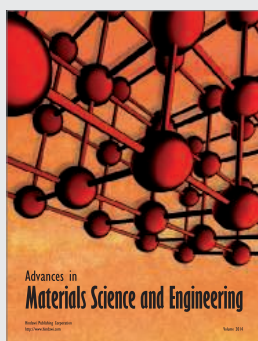
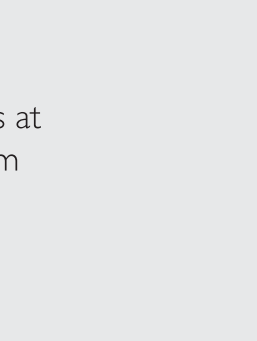
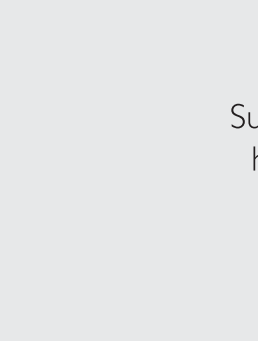
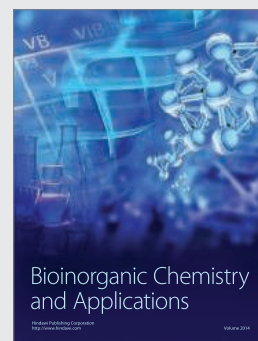
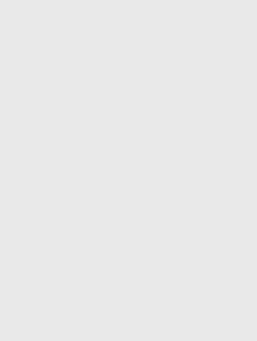
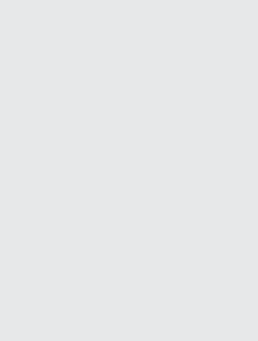
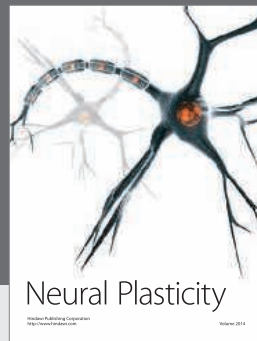
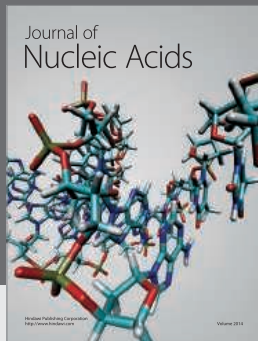
She was right. In that split second of terror, I realized what my real bar for accomplishment was. It wasn’t developing new theory, pioneering new methods, or getting research published in the best journals. I answered again: “You’re right. The bar for me is that when my career is winding down, because of my work, something is different in the water.” What I meant was, when I’m done I want to be able to say that I’ve had a mea-

asurable, significant impact on the environment I work to improve. “That’s more like it,” the professor responded.

This interaction haunted me for weeks because it compelled a follow-up question: “If this is my measure for success, what work must I do to achieve it?” I did some deep thinking and realized that my conservation work—not my academic research—was the work that would help me achieve those lofty goals.

I recalled seeing a presentation months before, in which the presenter asked his audience, “What got you into this field in the first place?” My answer: “I got in because I wanted to make things better.” But since then, my goals had shifted insidiously; my academic mentors were training new versions of themselves, and I had gone along. I had strayed from my original intention to use new knowledge to effect real-world change. And although I believe ardently that people can create change in a variety of work environments, I knew I needed to pursue a career that would ultimately be more rewarding. That’s why I decided to make the leap. ■

In April, Jack Kittinger became the director of Conservation International’s Hawaii Fish Trust program. For more on life and career issues, visit <http://www.sciencemag.org>.



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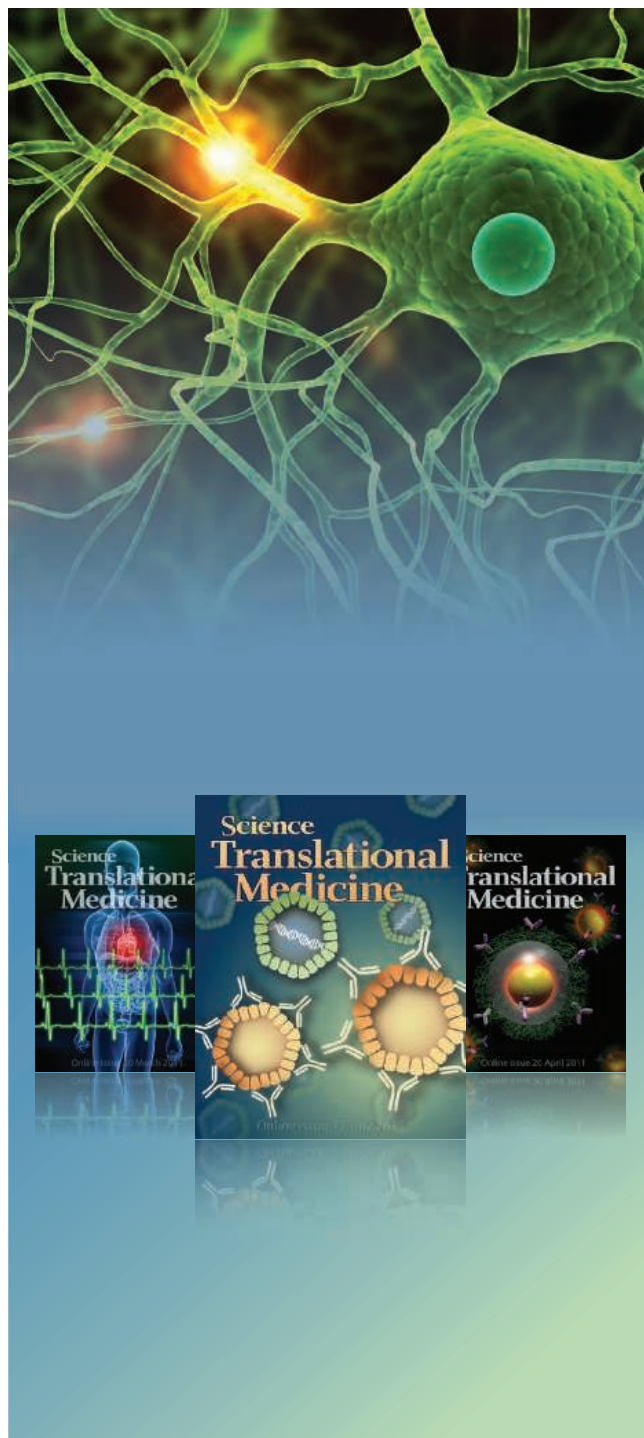
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D. Huh *et al.* (D. Ingber), *Sci. Transl. Med.* **5** 159ra147 (2012)

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S.L. Poon *et al.* (B. T. Teh), *Sci. Transl. Med.* **5** 197ra101 (2013)

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B. Sharma *et al.* (J.J. Elisseeff), *Sci. Transl. Med.* **5** 167 ra6 (2013)

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Bat man wins young scientist prize in Stockholm. Are you next?



Photo: Nancy Eweijn - UGA

The 2014 *Science* and SciLifeLab Prize is now open and we are looking for new bright ideas.

Are you like Daniel Streicker? Are you a recent PhD graduate eager to win a prize? Daniel is a true “bat man” having spent many hours in dark caves studying bats, to better understand how infectious diseases emerge and establish in new host species. It is for this work that Daniel was published in the journal *Science*, December 6, 2013, as the Grand Prize winner of the 2013 *Science* & SciLifeLab Prize for Young Scientists.

The journal *Science* and SciLifeLab have come together to recognize and celebrate excellence in PhD Research. The *Science* & SciLifeLab Prize has been established to support young scientists at the start of their career.

The four categories for the 2014 prize are: Cell and Developmental Biology; Genomics and Proteomics; Environment; and Translational Medicine. The Grand Prize winner will receive 25,000USD and the other three Category Prize winners will receive 3,000USD each. The Grand Prize winning essay will be published in *Science* and the Category Prize winners will be published in *Science* online.

The deadline for submissions is August 1, 2014.

For further details and to enter, please visit: www.sciencemag.org/scilifelabprize

*For over 130 years the journal *Science* has been the world's leading journal of original scientific research, global news and commentary.*

SciLifeLab is a collaboration among four universities in Stockholm and Uppsala, Sweden, and is a national center for molecular biosciences with focus on health and environmental research.

This prize is made possible with the kind support of the Knut and Alice Wallenberg Foundation.



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Science Careers

From the journal *Science*



ScienceCareers.org



The Institute for Geosciences at the Faculty of Geosciences/Geography at the Johann Wolfgang Goethe-University Frankfurt invites applications for a

Wilhelm Heraeus Endowed Professorship (W3) for Geophysics (with emphasis on Petrology)

Research will involve the investigation of physical, petrological and geochemical processes occurring in the Earth's mantle and crust. Possible directions of inquiry can include the role of volatiles in geodynamic processes, melting in different tectonic regimes, phase relations, recycling of crust into the mantle. Research should be complementary to the expertise existing in Frankfurt and focus on the application and development of physical, micro-analytical and/or geochemical methods with the potential coupling to high-pressure experiments to solve fundamental problems of the Earth's interior. Facilities at the Institute for Geosciences are perfectly suited for such investigations for further details, please see www.ifg.uni-frankfurt.de/48934943_ausstattung.

The successful candidate is expected to become actively involved in the Bachelor and Master of Geosciences programs, particularly in the fields of Geophysics and Petrology, additionally in Geochemistry and Mineralogy.

The successful candidate should be able to exhibit international competitive research experience, demonstrated by an accumulation of projects and publications. He/She will collaborate closely with already existing teams of the institute, possesses didactical skills as well as skills in personnel management. The applicant should provide a clear concept to establish interdisciplinary collaborative research projects with other faculty members in Geosciences and Physics, and possibly in other fields within the university.

The designated salary for the position is based on "W3" of the German university scale or equivalent. For further information regarding the general conditions for professional appointments, please see: <http://www.uni-frankfurt.de/aktuelles/ausschreibung/professuren/index.html>

Academics with an excellent record in research and teaching are invited to submit their applications accompanied by the usual documents (including CV, description of previous funding, publication list, teaching activities, statement of teaching and research interests) up to **August 1st, 2014** to the **Dean of the Faculty of Geosciences/Geography, Altenhöferallee 1, 60438 Frankfurt, Germany** or by e-mail to dekanat-geowiss@em.uni-frankfurt.de.

www.uni-frankfurt.de

SRI[®]JB

DIRECTOR

The Science & Resilience Institute at Jamaica Bay (New York)
Brooklyn College – CUNY

The newly formed Science & Resilience Institute at Jamaica Bay (SRIJB) seeks a dynamic and innovative scientist with demonstrated leadership, administrative, and research accomplishments to serve as its Founding Director. The position is expected to be filled by October 1, 2014.

The SRIJB is an exciting new initiative that will be both an important contributor of scientific knowledge and a major creator of opportunities for resilience practice in the socio-ecological systems in and around Jamaica Bay and beyond. In addition to its role in coordinating and leading science research in the field of resilience, it is expected to play an important role in public policy initiatives in the Bay through its respective public agency and stakeholder constituent arms. The former includes all local, state and federal agencies with jurisdiction in the area of Jamaica Bay; the latter includes non-profits and community organizations committed to the wellbeing of the Bay. Both of these entities are already in operation as components of SRIJB.

The Founding Director will oversee all research activities and will develop the capacity of the SRIJB to inform policymaking and resilience building projects in collaboration with all levels of government and with a wide range of social and environmental organizations. He or she will therefore be overseeing staff and/or subconsultants involved in the coordination of policy work, as well as more traditional program-based research staff.

The provisional offices for the SRIJB Directorate will be located at Brooklyn College, with plans to build a new permanent home on Jamaica Bay. The SRIJB Director will be a member of the Brooklyn College faculty and will be eligible for appointment as a full professor with tenure in a department in the natural or social sciences.

FOR MORE INFORMATION AND TO APPLY: Isaacson, Miller, a national executive search firm, has been engaged to assist with this important recruitment. Jane Gruenebaum and Pam Pezzoli are leading the search with Talia Greenwald. Inquiries, nominations, and applications should be directed in confidence to the firm through the website, www.imsearch.com/5055.

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UCI IRVINE

School of Medicine Department of Medicine Division of Basic and Clinical Immunology

The Department of Medicine at the University of California is seeking qualified candidates for an Associate or Full Professor, tenure track position in the Division of Basic and Clinical Immunology. This is a senate eligible 1.0 FTE position with potential for an Endowed Chair in Molecular Immunology. The successful candidate must possess a highly regarded track record of scholarly and academic achievement with current external peer-reviewed funding in a related field. The Division benefits from an outstanding scientific and clinical environment, including active collaborations with clinical and basic science departments and the Institute of Immunology. There are also opportunities for leadership, growth and synergy within the Department of Medicine.

Board certification in Immunology and eligibility for licensure in the State of California are required. M.D. and M.D./Ph.D. candidates.

To Apply: Please log into the UC Irvine Recruit system at: <https://recruit.ap.uci.edu/apply/JPF0232>. Applications will be considered until position is filled. Positions may start as early as **August 1, 2014**.

Contact information: Interested candidates should submit their CV and a letter describing their research interests, major achievements and future plans to: **Donald Forthal, Professor, Chief, Infectious Diseases, University of California, Irvine, 3044 Hewitt Hall, Irvine, CA 92617; dforthal@uci.edu; 949-824-3366.**

The University of California, Irvine is ranked first among US universities under 50 years old by Times Higher Education based on research and teaching excellence. The University of California, Irvine is an Equal Opportunity/Affirmative Action Employer committed to excellence through diversity. All qualified applicants will receive consideration for employment without regard to race, color, religion, sex, national origin, disability, age, protected veteran status, or other protected categories covered by the UC nondiscrimination policy.



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Postdoctoral positions and PhD studentship in
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Macroevolution, Community Ecology, Experimental and
Environmental Economics, and Bird Movement

A number of positions are available to work within an integrated terrestrial and marine research program addressing fundamental questions on the origin, maintenance, conservation and future of life and biological diversity on Earth.

Candidates should have track-record of publications, relevant analytical and data handling skills, and an ability to collaborate within an international research team. Competitive salaries and benefits are offered.

Full descriptions of individual positions are at
http://macroecology.ku.dk/opportunities_new/
Applications must be based on the full description of the positions, and submitted before **18 August 2014**. For enquiries about the program, contact **Professor Carsten Rahbek, crabek@snm.ku.dk**.

The center (<http://macroecology.ku.dk/>) is a long-term funded *center of excellence* with a cross-disciplinary research program addressing fundamental questions on the origin, maintenance, conservation and future of life and biological diversity on Earth. Researchers at the center currently represent 14 nationalities and the working language is English.



SciLifeLab

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SciLifeLab seeks an ambitious Director who can lead the scientific, technological and structural development of the center. We are looking for someone with extensive experience in both large-scale molecular biosciences and hypothesis-driven research; a person highly motivated to create a scientific, multidisciplinary and cutting-edge national center.

The position begins July 1st 2015 and is a six-year appointment as Director of SciLifeLab, which can be combined with a professorship at any of the four host universities. The appointment includes a significant research grant and start-up package.

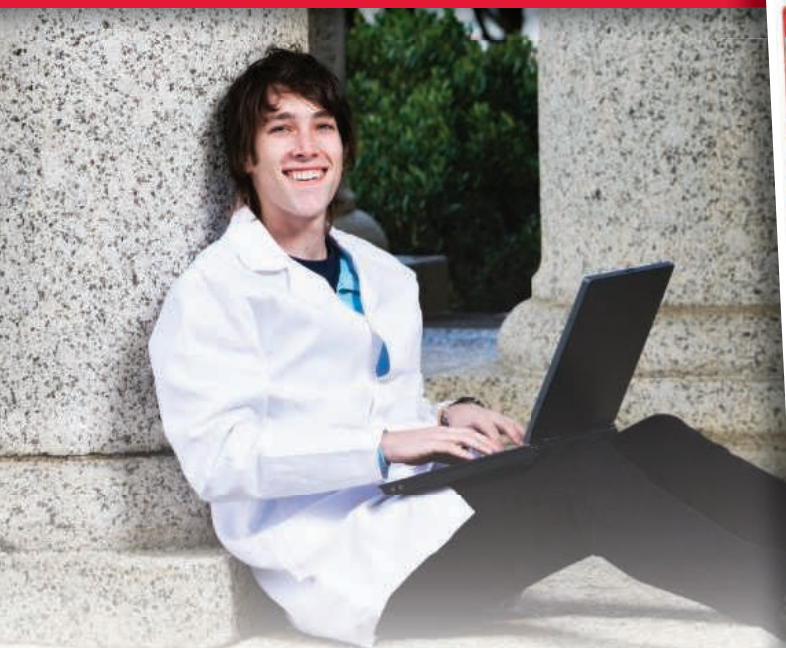
Learn more about the position at www.scilifelab.se/director

SciLifeLab (Science for Life Laboratory) is a national resource for large-scale bioscience research with a focus on health and environment. The center is run collaboratively between Karolinska Institutet, KTH Royal Institute of Technology, Stockholm University and Uppsala University. Today, approximately 1,500 people work at the center's two sites in Stockholm and Uppsala.



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Executive Director and Head of Science Programmes (2 posts)

Location: Paris, France

Closing date: 11 08 2014 (midnight CET)

The International Council for Science (ICSU) is recruiting two senior posts: an Executive Director, to lead implementation of the strategic goals and plans of the organization, manage its Secretariat and oversee day-to-day operations; and a Head of Science Programmes, to develop and deliver ICSU's science initiatives.

ICSU (www.icsu.org), founded in 1931, is a non-governmental organization dedicated to the promotion of science for the benefit of society. To accomplish its mission, ICSU facilitates, mobilizes and engages the knowledge and resources of the international science community represented by 31 Scientific Unions and 121 National Scientific Members (representing 141 countries) and 19 Interdisciplinary Bodies set up by ICSU to address specific scientific research areas.

ICSU has a central Secretariat of 18 staff located in Paris, plus three overseas Regional Offices in Africa, Asia and the Pacific, and Latin America and the Caribbean. The Paris office also currently hosts the interim secretariat for *Future Earth*.

Executive Director

The Executive Director reports to the elected ICSU Officers and the Executive Board. The incumbent is expected to: Act to achieve ICSU's mission, strategy and plans, and contribute to their formulation and development; Work with and seek synergy and harmony among all components of the ICSU membership: Scientific Unions, National Members, Associates and Interdisciplinary Bodies; Ensure effective links between ICSU and partners in the UN, governmental and non-governmental sectors; help raising funds in support of ICSU's programmes; and Manage the ICSU Secretariat and its staff in Paris and the Regional Offices around the world.

Head of Science Programmes

Reporting to the Executive Director, the Head of Science Programmes will manage the development and implementation of ICSU's scientific and science for policy initiatives, as defined in its Strategic Plan. The incumbent is also expected to: Line manage the Secretariat science staff; Take on project management responsibility for specific projects; and Support two of ICSU's policy committees (the Committee on Scientific Planning and Review and the Committee on Freedom and Responsibility in the conduct of Science), collaborating with the Chairpersons to help the committees meet ICSU's strategic needs.

For more details on the roles, required skills and experience, applications procedure and ICSU more generally, please visit <http://www.icsu.org/news-centre/jobs-at-icsu>



BIOLOGICAL SCIENCES SCHOLARS PROGRAM For Junior, Tenure-Track Faculty

The University of Michigan announces recruitment for the Biological Sciences Scholars Program (BSSP) to enhance its investigational strengths in the life sciences research programs. Now entering its 15th year, this Program has led to the recruitment of outstanding scientists in the areas of genetics, microbiology, immunology, virology, structural biology, biochemistry, molecular pharmacology, stem cell biology, cancer biology, physiology, cell and developmental biology, bioinformatics, and the neurosciences. The Program seeks individuals with PhD, MD, or MD/PhD degrees, at least two years of postdoctoral research experience and seeking their first appointment as an assistant professor. Candidates will show evidence of superlative scientific accomplishment and scholarly promise. Successful candidates will be expected to establish a vigorous, externally-funded research program, and to become leaders in departmental and program activities, including teaching at the medical, graduate, and/or undergraduate levels. Primary college and department affiliation will be determined by the applicant's qualifications and by relevance of the applicant's research program to departmental initiatives and focus. All faculty recruited via the BSSP will be appointed at the Assistant Professor level.

APPLICATION INSTRUCTIONS: Please apply to the Scholars Program through the BSSP website at: <http://bssp.med.umich.edu>. A curriculum vitae (including bibliography), a three page research plan, an NIH biosketch, and three original letters of support should all be submitted through the BSSP website. More information about the Scholars Program, instructions for applicants and those submitting letters of recommendation, and how to contact us is located on the BSSP web site: <http://bssp.med.umich.edu>. The deadline for applications is **Friday, October 17, 2014**.

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Science Positions



Idaho National Laboratory's Energy & Environment Science & Technology (EEST) Directorate is seeking outstanding, highly creative and motivated early to mid-level career professionals to join our multi-disciplinary research teams. The EEST Directorate is INL's principal multi-mission organization focused on research to advance clean energy systems, advanced transportation, advanced process technology and related sciences. Accomplished individuals with research interest in the following areas are strongly encouraged to apply:

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Idaho Falls is conveniently situated near many national treasures such as Yellowstone National Park, Teton National Park, Jackson Hole, WY and Sun Valley. For more information about the area, please visit www.visitidahofalls.com and www.visitidaho.org. To apply and learn more about current opportunities, visit our website at www.inl.gov.

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Send curriculum vitae and recent publications to: Erol Fikrig M.D., Investigator, Howard Hughes Medical Institute, Yale University School of Medicine, Section of Infectious Diseases, P.O. Box 208022, New Haven, CT 06520-8022 or e-mail: erol.fikrig@yale.edu. Yale University is an Affirmative Action/Equal Opportunity Employer. Applications from women and minorities are encouraged.

ASSISTANT OR ASSOCIATE PROFESSOR

The College of Pharmacy, Washington State University (WSU) in Spokane, invites applications for a tenure-track full-time faculty position at the rank of Assistant or Associate Professor (commensurate with experience) in the Section of Experimental and Systems Pharmacology. The successful candidate is expected to maintain an active, extramurally funded research program in a relevant area (broadly defined), including pharmacology, physiology, therapeutics and toxicology; to mentor graduate and PharmD students and fellows; and to teach in the professional and graduate curricula on topic(s) in the potential areas of pathophysiology, pharmacology, therapeutics, medicinal chemistry, or other areas appropriate to the candidates' background. Required Qualifications: Applicants must have an earned advanced degree (M.D., PharmD., or Ph.D. in Pharmacology, Pharmaceutical Sciences, or a related discipline) before employment start date; All But Degree will be considered but all Ph.D. degree requirements must be completed prior to an offer being extended. Candidates at the level of Assistant Professor must possess a track record of accomplishment that demonstrates the potential to become an outstanding scholar and educator. Candidates considered at the rank of Associate Professor must possess a record of accomplishment demonstrating outstanding scholarly and educational activities, with a nationally recognized, extramurally funded, research program. Preferred Qualifications: Applicants with a research focus involving pharmacokinetic/pharmacodynamic modeling and simulation and discovery and development of drugs for special or underserved populations are especially encouraged to apply.

Screening of applications will begin September 01, 2014 and will continue until a suitable candidate is identified. To apply, visit [website: http://www.wsujobs.com](http://www.wsujobs.com). Applications must include the following materials: (1) A letter of application describing your professional goals, relevant academic preparation, and experience in research and teaching; (2) A current curriculum vitae; and (3) Name, title, organization, telephone number, and e-mail address for five people willing to serve as employment references. To apply and see complete position description visit [website: http://www.wsujobs.com](http://www.wsujobs.com). WSU is an Equal Opportunity/Affirmative Action/ADA Educator and Employer.

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POSITIONS OPEN

RESEARCH ASSOCIATE The University of North Carolina at Charlotte Bioinformatics Services Division

The Bioinformatics Research Center at University of North Carolina (UNC) at Charlotte invites applications for a Research Associate (position #3978). UNC-Charlotte's Bioinformatics Research Center has the leadership role in bioinformatics for the nearby North Carolina Research Campus (NCRC) at Kannapolis ([website: http://www.ncresearchcampus.net](http://www.ncresearchcampus.net)). The successful candidate will have expertise in the analysis of high throughput sequencing data and the analysis of sequence variations (SNPs, indels, rearrangements, and copy number). They will have experience with the latest tools and practices for variant analysis. They will have used these technologies in their own or their collaborators research. Experience with cancer samples a plus. The successful candidate will have experience interpreting this data and understanding the biological significance. They will also have command line experience on Linux/Unix and be fluent in at least one scripting/programming language (i.e., Perl, Python, Java, etc.). The candidate should also have outstanding communication skills, be able to communicate with biologists, demonstrate good teamwork, and have a publication record. This position requires a Ph.D. or Master's degree in bioinformatics, genetics, molecular biology, or related degree specializing in genetic variation. The candidate must also be a U.S. citizen or Green Card holder to comply with U.S. export policies. Applications must be made electronically at [website: http://www.jobs.uncc.edu](http://www.jobs.uncc.edu) (position #3978) and should include vitae, at least three references, and a letter of interest. The UNC-Charlotte is an Equal Opportunity Employer/Affirmative Action Employer and an ADVANCE Institution. For additional information, please visit our [website: http://www.bioinformatics.uncc.edu](http://www.bioinformatics.uncc.edu).

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POSITIONS OPEN

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The College of Pharmacy, Washington State University (WSU) on the Riverpoint campus in Spokane, invites applications for a full-time, tenure-track faculty position at the rank of Assistant Professor (commensurate with experience) in the Section of Experimental and Systems Pharmacology. Applicants must have an earned Ph.D. in biostatistics, epidemiology, or a related field (such as public health, biomathematics, biometrics, or quantitative systems pharmacology) before employment start date. All But Degree will be considered but all Ph.D. degree requirements must be completed before an offer is extended; an established peer-reviewed publication record; must possess a track record of accomplishment to demonstrate the potential to obtain extramural research support. The applicant must have expertise in the use of SAS or R/S-PLUS. Further, the successful applicant should possess a tracked record of accomplishment that demonstrates the potential to become an outstanding scholar and educator. Preferred qualifications for this position include evidence of tracked record supporting the design, implementation and analysis of clinical trials; health outcomes, economic evaluation and comparative effectiveness research; statistical methodology for analysis of epidemiological data including electronic health records; or systems biology and population pharmacokinetic/pharmacodynamic modeling. Duties include: teaching in both the professional (PharmD) and graduate (Ph.D.) curricula, on topics of biostatistics, study design, epidemiology, healthcare outcomes, ancillary areas, methodological research in the candidate's area of specialization, and collaborative pharmaceutical/biomedical research.

Screening of applications will begin July 1, 2014 and will continue until a suitable candidate is identified. To apply, visit [website: http://www.wsujobs.com](http://www.wsujobs.com). Applications must include the following materials: (1) a letter of application describing your professional goals, relevant academic preparation, (2) a current curriculum vitae; and (3) name, title, organization, telephone number, and e-mail address of a minimum of five people willing to serve as employment references. To apply and see complete position description visit [website: http://www.wsujobs.com](http://www.wsujobs.com). WSU is an Equal Opportunity/Affirmative Action/ADA Educator and Employer.

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WWSC
WALLENBERG WOOD
SCIENCE CENTER

Three Assistant professors & one Associate professor to Wallenberg Wood Science Center

Thanks to generous support from the Knut and Alice Wallenberg Foundation, WWSC can now announce four positions intended for younger scientists:

Three assistant professorships with placement at Chalmers:

- Plant cell wall enzymology, ref no 20140194, www.chalmers.se
- Forest Products and Chemical Engineering, ref no 20140193, www.chalmers.se
- Wood biopolymer chemistry, ref no 20140192, www.chalmers.se

The application dead-line is September 15, 2014.

One associate professorship with placement at Stockholm University:

- Materials chemistry, with focus on biopolymer-based functional materials, ref no SU FV-1501-14, www.su.se/english/about/vacancies

The application dead-line is August 31, 2014

In addition to financial support during the first 4 years, WWSC also offer funding for a post-doc position for 2 years for the selected candidate.

Wallenberg Wood Science Center is a research center with a focus on new materials from trees. About 60 researchers from five different Universities (KTH, Chalmers, Stockholm University, Umeå University and Luleå Technical University) collaborate in a research environment of highest international standing. The program is on green processes and biobased materials and devices.



CHALMERS
UNIVERSITY OF TECHNOLOGY



nature

Chief Editor, *Nature Neuroscience*

The Nature Publishing Group is looking for a Chief Editor for *Nature Neuroscience*, the leading biomedical research journal devoted to publishing the latest and most exciting advances in neuroscience. The journal covers all areas of neuroscience, including molecular, cellular, systems and cognitive neuroscience, as well as psychophysics, computational modeling and diseases of the nervous system. The position of Chief Editor is a senior appointment, reporting to the Executive Editor, and is backed by a strong commitment to excellence and investment in resources. This is an exciting opportunity to join the world's leading scientific publishing company and to be involved with the development of a prestigious journal. The Chief Editor is responsible for leading a skilled and enthusiastic editorial team and for developing the editorial content of the journal, both in print and online.

To meet these challenging tasks, the ideal candidate will have intellectual vision as well as strong leadership qualities. She or he should have a PhD in neuroscience (any area), have excellent scientific judgment, and a broad knowledge and understanding of basic research in the life sciences. The ideal candidate will have a strong research background and publication record while previous editorial and/or managerial experience is an additional advantage. A key aspect of the job is interacting with the scientific community and attending international conferences. The successful candidate must, therefore, be dynamic and outgoing, be prepared to travel, and have excellent interpersonal skills.

The position will be based in Nature Publishing Group's office in New York, and accordingly candidates must be able to demonstrate the right to live and work in this location. The terms and conditions are highly competitive, reflecting the importance and responsibilities of the role.

To apply, please go to <https://home.eease.adp.com/recruit/?id=9836941> and submit a cover letter stating your suitability for this post, salary expectations, a current CV, and a statement that encapsulates your future vision for Nature Neuroscience. All applicants will be reviewed upon receipt with a close date of **July 18, 2014**.

To learn more about our company, please visit our web site at www.nature.com.

Nature Publishing Group is an Equal Opportunity Employer.



Agricultural Scientists Recruitment Board

Krishi Anusandhan Bhawan-I, Pusa
New Delhi - 110012 (INDIA)

ADVT. NO. 02/2014 (PART)



INVITING APPLICATION FOR DIRECTORS

Agricultural Scientists Recruitment Board (ASRB), an independent recruiting agency for Indian Council of Agricultural Research (ICAR), proposes to fill up two leadership level Senior Research Management Positions (RMP), namely **Director, National Institute of Biotic Stress Management (NIBSM), Raipur, Chhattisgarh** and **Director, Indian Institute of Agricultural Biotechnology (IIAB), Ranchi, Jharkhand**. Requirements for these positions and procedure for applying including the application form, as contained in the detailed Advertisement No. 02/2014, can be accessed/downloaded from ICAR/ASRB websites viz. www.icar.org.in (at 'Quick Link'-Employment-ASRB Advertisements) and www.asrb.org.in (at the link 'Vacancy'-Detailed ASRB-Advt. No. 02/2014) respectively. The above positions are at Item No. 82 and 83 respectively in the detailed Advertisement No. 02/2014. ASRB/ICAR may, in its discretion, relax any or all the conditions in case of exceptionally qualified and eminent scientists in the required research and development fields.

PRUNIT/ICAR/2014

N. S. Randhawa, Secretary